Sagitta setosa predation on Calanus helgolandicus in the English Channel

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We used a long-term monitoring data set at station L4 (1988–2004), Western English Channel, to assess the predation pressure by the chaetognath Sagitta setosa on the copepod Calanus helgolandicus. Maximum abundances of Calanus helgolandicus are correlated with years when Sagitta setosa and the Siphonophore, Muggiae atlantica, abundances are low, between February and June. As a significant correlation does not necessarily imply a prey–predator interaction, we analysed the gut content of S. setosa to investigate the presence or absence of Calanus helgolandicus in the chaetognath diet. Molecular analysis of the gut content of three Sagitta each month during a year shows that C. helgolandicus is present in the diet of S. setosa throughout the year. Estimates of S. setosa minimal predation pressure suggest that up to 19% of the C. helgolandicus population can be removed. Peaks of S. setosa follow peaks of Calanus helgolandicus total egg production. We suggest that S. setosa is an important predator of C. helgolandicus at station L4 and might be an important influence on its population dynamics.

KEYWORDS: Calanus helgolandicus; Chaetognath; Sagitta setosa; predation; long-term series; English Channel

INTRODUCTION

At northern latitudes, long-term trends of zooplankton communities have been shown to be closely linked to the North Atlantic Oscillation or the position of the Gulf Stream North Wall (Clark et al., 2003; Reid et al., 2003). Changes in temperature and salinity result in changes in copepod assemblages (e.g. Beaugrand et al., 2002), but several studies have also indicated that long-term trends could be significantly related to biotic parameters (Frid et al., 1994; Huliselan, 1995; Clark et al., 2003). Zooplankton community mortality is difficult to assess, but is thought to be an important parameter affecting population dynamics as it could be more important than growth in controlling abundance and biomass of a population (Uye et al., 1992; Ohman and Hsieh, 2008).

The role of zooplankton predator–prey interactions in structuring plankton communities is still poorly understood despite a large number of studies on planktivorous fish (i.e. Bacha and Amara, 2009; Prokopchuk, 2009), bird (i.e. Fortier et al., 1994; Tremblay et al., 2006) and invertebrate predation on copepods (i.e. Hirota, 1974; Purcell, 1981; Yen, 1985; Øresland, 1987; Drits andUtikina, 1988). The main reason is probably the difficulty of experimenting on or culturing and maintaining some of these predators in the laboratory, especially vertically migrating gelatinous organisms.

At station L4, Calanus helgolandicus is the predominant Calanus species and C. finmarchicus only occurs in very low numbers at particular times of year (Russell, 1951; L4 database at www.westernchannelobservatory.org.uk/). In contrast, Calanus helgolandicus is present throughout the
year, but little is known about predation on this species (Irigoien and Harris, 2003; Hirst et al., 2007). Mesopelagic fishes (Prahl et al., 1985; Bagoeien et al., 2001) together with krill (Bagoeien et al., 2000) and basking sharks (Sims and Merrett, 1997) are presumed to feed on C. helgolandicus in other areas. Cannibalism has also been suggested as a potential source of mortality (Bonnet et al., 2004). However, predation measurements on C. helgolandicus are scarce and the potential impact on population dynamics rarely documented at station L4 (Hirst et al., 2007).

Chaetognaths are a major component of the zooplankton community and can represent 10–30% of the zooplankton biomass in the world oceans (Reeve, 1970; Bone et al., 1991). At L4, a single species S. setosa is present. Chaetognaths are considered important predators which can structure the population dynamics of copepods (Davis, 1984; Sullivan and Meise, 1996; Clark et al., 2003). While many authors have managed to identify the prey encountered in the chaetognath guts from identification of prey mandibles, chaetognath hooks and other hard or special prey parts, most of the studies acknowledge that 35–50% of the gut contents of S. setosa are unidentifiable prey items (Pearre, 1980; Øresland, 1987; Duró and Saiz, 2000; Tönnesson and Tiselius, 2005). Usually, the proportion of unidentifiable prey increases as chaetognath size decreases, probably because small ones will have eaten smaller and more quickly digested prey.

The grasping and swimming behaviour and the vibration sense of Sagitta seems to be specialized for catching copepods (Newbury, 1972) and predation pressure of chaetognaths on copepods is well established. Highly digested copepod remains in chaetognath guts or early developmental stages are difficult to identify and in particular nauplii remain un-identified in gut contents. Hence, the predation impact of chaetognaths on copepod populations may be underestimated.

We developed two approaches to study the interaction between Calanus helgolandicus and its predator Sagitta setosa: (i) we looked at the long-term pattern of prey and predator abundances at station L4 (from 1989 to 2003), and (ii) we identified, using a molecular approach, the presence of C. helgolandicus in S. setosa gut contents.

**METHOD**

**L4 time series**

Station L4 (50°15’N, 04°13’W) is located 10 nautical miles south-west of Plymouth in the Western English Channel. Zooplankton sampling at this station started in 1988 and has been incorporated into a weekly time-series sampling programme (see http://www.westernchannelobservatory.org.uk/l4/data). Samples were always collected at mid-morning by vertical net hauls (WP2 net, 200 µm) from the bottom (~55 m) to the surface and fixed in 5% formalin. Samples were then counted for major taxonomic groups as well as identifying some groups, particularly copepods, to species level. Large organisms were identified in large subsamples taken with a Folsom splitter, whereas splits taken with a stempel pipette allowed identification and counts of smaller organisms. Sub-samples contained around 100 individuals. Samples were sub-sampled twice and the results averaged.

**Initial data manipulation**

Of the 832 weeks in the 1988–2003 series, there were 157 missing data points when sampling was not carried out. Of these missing data points, 129 were filled by simple linear interpolation between adjacent sampled weeks (i.e. when sampling was missed in a single week but sampling was carried out in the weeks either side). The remaining missing data points were interpolated from the long-term (all sampled years) mean abundance for a particular week adjusted proportionately to abundance for the remainder of that year.

**Molecular analysis of the gut contents of Sagitta setosa**

Sagitta setosa has a relatively short digestion time (Table I; Mironov, 1960; Duró and Saiz, 2000 and references therein; Tönnesson and Tiselius, 2005) and seems very often to have empty guts (Personal observation; Wimpenny, 1937; Rakusa-Suszczewski, 1969). From January 2005 to January 2006, we detected the presence of Calanus helgolandicus in three Sagitta setosa guts using molecular techniques. A polymerase chain reaction (PCR)-based approach was used to determine whether Calanus DNA can be detected in the gut of S. setosa. Studies on carnivorous insects and other organisms have demonstrated that PCR-based methods for detecting prey DNA are highly effective and versatile (Symondson, 2002). Such techniques have recently been used for marine organisms (Nejstgaard et al., 2003; Blankenship and Yayanos, 2005; Vestheim et al., 2005; Durbin et al., 2008; Nejstgaard et al., 2008; Simonelli et al., 2009) showing that DNA from consumed prey is not completely degraded during digestion and therefore can be amplified via PCR from both gut contents and faecal pellets, potentially providing an excellent approach to investigating predator–prey relationships of marine zooplankton. We recognize that this approach is
### Table I: Review of the chaetognath Sagitta setosa feeding rates on copepods

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Species</th>
<th>Prey</th>
<th>NPC (prey ind.⁻¹)</th>
<th>DT (h⁻¹)</th>
<th>FR (prey day⁻¹)</th>
<th>Specific daily ration (dry wt basis)</th>
<th>T (°C)</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetognath</td>
<td>S. setosa</td>
<td></td>
<td>0.219</td>
<td>2.3</td>
<td></td>
<td>14</td>
<td>14</td>
<td>Gullmarsfjord, Sweden</td>
<td>Øresland (1987)</td>
</tr>
<tr>
<td></td>
<td>S. setosa</td>
<td></td>
<td>0.250</td>
<td>6</td>
<td></td>
<td>16</td>
<td>6</td>
<td>Bay of Sevastopol</td>
<td>Mironov (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nauplii</td>
<td>0.085ₐ</td>
<td>1</td>
<td>2.04</td>
<td>1.68–0.072 (larvae-adult)</td>
<td>11.5</td>
<td>Mediterranean Sea</td>
<td>Duró and Saiz (2000 and references therein)</td>
</tr>
<tr>
<td></td>
<td>S. setosa</td>
<td></td>
<td>0.28 to 0.91</td>
<td>5</td>
<td></td>
<td>9 to 16</td>
<td>9</td>
<td>Gullmar fjord, Sweden</td>
<td>Tönnesson and Tiselius (2005)</td>
</tr>
<tr>
<td></td>
<td>S. setosa</td>
<td>Copepods, nauplii, chaetognaths, appendicularians</td>
<td>0.245ₐ</td>
<td>2.88ₐ</td>
<td>2.04ₐ</td>
<td>0.062</td>
<td>15</td>
<td>Laboratory experiment</td>
<td>Kuhlmann (1976, 1977)</td>
</tr>
</tbody>
</table>

NPC, number of prey per individual; DT, digestion time (h⁻¹); FR, daily feeding rate (number of prey ate per individual per day).

ₐThe feeding rate (FR, prey day⁻¹) was calculated using Bajkov’s equation (Bajkov, 1935): FR = NPC × 24/DT, DT was estimated from temperature according to Ohman (1986): DT = 10.48 e⁻⁰.086T, where T is the temperature in degree Celsius.

ₐBased on calculations using data from Feigenbaum and Maris (1984).
only qualitative, not quantitative, however determining if *C. helgolandicus* is part of the diet of *S. setosa*, will allow a better interpretation of abundance trends of both organisms from the long-term series.

Up to 400 *Sagitta setosa* (Table II) were fixed in 95% ethanol every week. Ingested material, from three *S. setosa* adults with a full gut, was dissected out with sterilized forceps and scalpel from the posterior two-thirds of the gut. Material from the anterior third of the gut normally appeared to show some resemblance to the ingested organism (e.g. copepod, larvae and egg), material from the centre third of the gut was unrecognizable but could still be found in large fragments, material from the posterior third of the gut was completely broken down. Ingested material from the anterior third of the gut was not used for molecular analysis due to the possibility of false feeding in the crowded conditions of the net and cod end leading to contamination with prey which would not ordinarily be part of the diet (Pearre, 1974; Øresland, 1987; Alvarez-Cadena, 1992; Kehayias, 2003; Kehayias et al., 2005). For each month from January 2005 to January 2006, ingested material recovered from the posterior 2/3 of the gut was removed from three individual *S. setosa* (39 individuals in total) under a dissecting light-microscope. The material was split into three aliquots in order to perform triplicate PCR reactions and each aliquot was rehydrated in 30 μL of MilliQ water in a 1.5 mL centrifuge tube at 4°C for 6–12 h. Following rehydration 10 μL of 5× Hot Start Taq DNA polymerase buffer (Promega UK, Ltd) was added.

The material was then homogenized using a 21G needle inserted into a pellet pestle homogenizer (Anachem Ltd) and incubated overnight at 4°C. After incubation, the remaining reaction components of the PCR reaction were added [5 μL 2 mM dNTPs, 2.5 μL each of primers 16SAR and 16SB2R (10 μMolar), and 2.5 U GoTaq® Hot Start DNA polymerase (Promega UK Ltd)]. The amplification primers used were the universal forward primer 16SAR (5’-cgcctgtttaacaaaaacat-3’; Palumbi and Benzie, 1991) and the *Calanus* specific reverse primer 16SB2R (5’-attaacatcgaggtcacaaac-3’; custom designed from *Calanus* spp. sequence data, Lindeque et al., 1999). Amplifications were carried out in a thermal cycler (Unocycler, VWR). The cycling parameters included an initial denaturation step at 94°C (5 min) followed by 40 cycles of 45°C (2 min), 72°C (1 min) and 94°C (1 min). A final annealing phase at 45°C (2 min) was followed by an extension phase at 72°C (5 min) and storage at 4°C until use. A positive control of a non-ingested identified *Calanus helgolandicus* and a no-template negative control were included. Two false positive controls were also performed using (i) a portion

| Table II: Characteristics and numbers of samples analysed by PCR and estimates of the FCR and predation pressure on *C. helgolandicus* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number of *S. setosa* sorted | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 | 302 |
| Number of *S. setosa* with material in their gut | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Number of *S. setosa* with material in their posterior 2/3 gut | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Number of *S. setosa* with material in their posterior 2/3 gut analysed by PCR | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Number of *S. setosa* from row above showing a positive PCR | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Number of positive PCR | 1118 | 1.133 | 8.006 | 14.232 | 3.488 | 4.440 | 2.521 | 2.197 | 0.725 | 0.373 | 0.349 | 1.059 |
| Total FCR (% of *S. setosa* in the total sample with *C. helgolandicus* in their posterior 2/3 posterior gut) | 0.349 | 0.373 | 0.349 | 0.373 | 0.349 | 0.373 | 0.349 | 0.373 | 0.349 | 0.373 | 0.349 | 0.373 | 0.349 | 0.373 | 0.349 |
| Predation pressure on *C. helgolandicus* (% of *S. setosa* in the total sample with *C. helgolandicus* in their posterior 2/3 posterior gut) | 4.6 | 2.5 | 5.6 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
of *S. setosa* as template and (ii) a mixture of potential *Sagitta* prey organisms found at station L4 as template. The copepod, other than *Calanus helgolandicus*, contribution is specified in terms of the total copepod abundance for those > 1%: *Oncaea* spp. (26.9%), *Pseudocalanus parvus* (20.3%) (Tönnesson and Tiselius, 2005), *Pseudocalanus elongatus* (12.5%) (Tönnesson and Tiselius, 2005), *Temora longicornis* (7.8%) (Oresland, 1987), *Gryphea anglica* (2.6%) and *Euterpeina acutifrons* (1.2%). Other potential prey were also present: echinoderms, molluscs, fish (Mironov, 1960), polychaetes (Mironov, 1960) and decapod larvae as well as cladocerans (Mironov, 1960) and fish eggs. Amplification efficiency was checked by analysing a 5 μL aliquot of each reaction by agarose gel electrophoresis (1.5%). A random selection of six successful amplification products was cleaned by ethanol precipitation, cloned using pGEM-T Easy Vector System I kit (Promega UK Ltd) and sequenced using Applied Biosystems chemistry on an ABI 3100 sequencer to confirm their identity.

This molecular approach is only qualitative, however we used these results to determine the minimum predation pressure of *S. setosa* on *C. helgolandicus* and estimated the percentage of *Calanus helgolandicus* population removed in this case.

Feeding activity was expressed as FCR (percentage of *Sagitta setosa* containing *C. helgolandicus* in their gut) and NPC (number of *C. helgolandicus* per chaetognath). A minimum value of 1 was fixed in our study when the presence of *Calanus helgolandicus* in the gut was positive as the number of *C. helgolandicus* in the gut could not be estimated.

The prey located in the foregut were not used for the calculation of FCR and NPC values because they might have been artefacts due to cod-end feeding.

The ingestion rates (*I*, prey ingested per chaetognath and per day) were calculated according to Feigenbaum’s equation (Feigenbaum, 1991):

\[ I = \text{NPC}/\text{DT} \times 24 \]

Where DT is digestion time (in hours). DT was estimated from temperature according to Ohman (Ohman, 1986): \[ \text{DT} = 10.48 e^{-0.0867T}, \] where *T* is the temperature in degree Celsius.

Predation pressure (PP in %) on *C. helgolandicus* standing stock was estimated:

\[ \text{PP} = I \times \text{FCR} \times \text{Nt} \times 100/\text{Ne} \]

with *Nt*, the total abundance of *Sagitta setosa* (ind. m\(^{-3}\)), and *Ne*, the total abundance of *Calanus helgolandicus* (ind. m\(^{-3}\)). FCR was estimated as follows:

\[ \text{FCR} = \text{Ng}/\text{Ns} \times P \]

where *Ns* is the number of *Sagitta setosa* sorted from the samples, *Ng* the number of *S. setosa* with material in their 2/3 posterior gut, *P* the probability of finding *Calanus helgolandicus* in *S. setosa* gut. The value of *P* was estimated from the aliquot of three organisms taken for PCR analysis each month (*P* = 1/8 when no *C. helgolandicus* was detected in the gut or when all the three organisms contained *C. helgolandicus*, *P* = 3/8 when one or two of the samples out of three contained *C. helgolandicus*).

**RESULTS**

**Seasonal cycles**

The mean annual cycle of *Calanus helgolandicus* and potential predator abundance identified from the WP2 net sampling over the L4 time series are presented in Fig. 1. The species considered here contribute at least 5% of the total numerical abundance at L4. From February to May, predator abundance is relatively low and Chaetognaths and Hydromedusae represent, respectively, 14.15 and 11.70% of the total predator abundance. These predators as well as the *C. helgolandicus* seasonal cycle show a unimodal distribution. The most abundant predators are siphonophores, hydromedusae and chaetognaths. At L4, predator abundance begins to increase at the beginning of April (Fig. 1). Predators appear when *C. helgolandicus* is abundant and show different mean maximum abundance over the year. Hydromedusae are abundant from June to August (maximum = 59 ind. m\(^{-3}\)), siphonophores, from July to October (maximum = 118 ind. m\(^{-3}\)) and chaetognaths reach their maximum abundance in September and October (maximum = 73 ind. m\(^{-3}\)). *Calanus helgolandicus* abundance increases strongly from January to June when it reaches its maximum (maximum = 134 ind. m\(^{-3}\)) before declining over the winter. Amphipods, fish larvae and euphausiids show very low abundances (<10 ind. m\(^{-3}\)), with fish larvae mainly present during the first half of the year and amphipods from June onwards.

**Long-term patterns**

Figure 2 shows the long-term pattern of *C. helgolandicus* potential predators at L4. On average, no distinct long-term trend can easily be observed in predator
abundance. Most of the predators appear in the second half of the year apart from fish larvae which tend to be present in the first half and euphausiids which are very rare. Consequently, the long-term impact on *Calanus helgolandicus* population dynamics was considered in the rest of our study concentrating only on chaetognaths, siphonophores and hydromedusae. There is clear evidence of inter-annual differences in the abundance and relative dominance of the pelagic predators at L4 over the course of this study. *Calanus helgolandicus* long-term abundance also shows some high inter-annual variability with a change in the timing of the maximum abundance: week 22 in 1988, week 18 in 1995 and weeks 26 and 32 in 2002 (Fig. 3). However, *C. helgolandicus* is generally quite abundant throughout the year, with low abundances observed in winter only.

*Calanus helgolandicus* average monthly abundance shows no relationship with its maximum abundance during the year other than in June, July and August (Fig. 4). However, there is a significant correlation between the difference in abundance of *C. helgolandicus* from February to May (i.e. their increase in abundance over this period) and their maximum annual abundance ($r = -0.463, P \leq 0.041$). This result indicates that the...
variations in abundance from February to May are critical in determining the maximum annual abundance reached.

Standardized time series of the abundances of predators and *C. helgolandicus* show some significant relationships at station L4 (Fig. 5). Abundance of chaetognaths and siphonophores is significantly correlated to the *Calanus helgolandicus* population (*P* < 0.05), whereas the abundance of hydromedusae is not.

**Molecular analysis of *Calanus helgolandicus* presence in *Sagitta* gut contents**

Of the 39 samples picked from the posterior two-thirds of the gut, 17 produced an amplification product of the expected length (408 bp) and the same size as the positive control (Fig. 6). There was no discrepancy between the triplicate reactions, with either the PCR working for all three replicates or not working for any of the three replicates. The negative control contained no amplification product. The false positive control of *Sagitta* template contained no amplification product and the false positive control consisting of a mix of other potential prey organisms resulted in a smear with no definable amplicon (data not shown). Sequencing of the successful amplicons confirmed their identity to be *Calanus helgolandicus*, showing >99.3% homology when compared with existing *C. helgolandicus* sequence data (accession number: AJ31158).

**DISCUSSION**

**Long-term pattern**

Analysis of the long-term data set has shown that only the interaction between *Calanus helgolandicus* population dynamics and siphonophore and chaetognath abundance was significant (Fig. 5). From Cushing’s equations (Cushing, 1983), we can suggest that the other predator densities were too dilute to affect the abundance of the *C. helgolandicus* population. Chaetognaths and siphonophores are well known as potential predators on copepod populations (e.g. Purcell, 1981, 1982; Feigenbaum and Maris, 1984), but only a few studies are based on long-term series data (e.g. Ohman, 1986; Clark et al., 2003). For example, Ohman (Ohman, 1986) related the variability in the annual mortality of *Pseudocalanus* sp. in Dabob Bay, Washington to the abundance of predators such as *Sagitta elegans*, but also the omnivorous euphausiid *Euphausia pacifica* and the predatory copepod *Euchaeta elongata*. Similarly, Clark et al. (Clark et al., 2003) suggested that predation by *Sagitta* on omnivorous zooplankton taxa plays an important role.

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**Fig. 3.** Long-term abundance and seasonality of *Calanus helgolandicus* at station L4.

**Fig. 4.** Correlation coefficients calculated over the whole L4 time series between *Calanus helgolandicus* abundance for each month and its total maximum abundance during the year. Grey bars indicate significant correlations (*P* ≤ 0.05).
in controlling the long-term dynamics of the coastal central-west North Sea zooplankton community. Our results show that both chaetognath and siphonophore abundance from February to June are significantly inversely correlated with maximum abundances of *C. helgolandicus* throughout the time series suggesting that both predators are responsible for structuring *C. helgolandicus* population dynamics.

At station L4, siphonophores are represented by a single species *Muggiae atlantica*. To our knowledge, there is only one study reporting *Muggiae atlantica* feeding rates on copepods (Purcell, 1982). However, though Purcell (Purcell, 1982) determined a daily *in situ* prey consumption ranging from 5.5 to 10.5 prey siphonophore$^{-1}$ day$^{-1}$, she did not estimate the predation pressure on the copepod population. However, such high consumption rates reveal the potential top down control by siphonophores on copepod populations, especially as *M. atlantica* is the most abundant predator from June to October at L4.

Because of the limited information on siphonophore feeding ecology and as *S. setosa* abundance from February to June showed the best relationships with *C. helgolandicus* maximum abundance throughout the L4 time series (Fig. 5), we focused on the interaction between these two last species.

**The contribution of *Calanus helgolandicus* to the *Sagitta setosa* diet**

Reeve (Reeve, 1970) estimated that chaetognath biomass represent about 30% of that of copepods in the world oceans. He suggested that most of the energy converted to biomass by copepods was transferred to higher trophic levels *via* chaetognaths (Feigenbaum, 1991). While several studies have focused on feeding mechanisms (Horridge and Bolton, 1967; Newbury, 1972; Feigenbaum and Reeve, 1977; Reeve, 1980) or food selection (Reeve and Walter, 1972; Pearre, 1973, 1974; Nagasawa and Marumo, 1976; Drits, 1981; Kimmerer, 1984; Baier and Purcell, 1997; Marazzo et al., 1997; Saito and Kiorboe, 2001), diets have mostly been determined from gut contents of preserved specimens because of the difficulty of keeping and feeding most chaetognath species in the laboratory (Fraser, 1969).

Attempts to correlate Chaetognath feeding in nature with zooplankton availability have been difficult for several reasons: (i) Chaetognaths do not necessarily feed at the depths they are caught (Pearre, 1973, 1974; Gibbons, 1993). Predator rate attacks are highly density-dependent and thus sensitive to prey dispersion in the water column (vertical distribution), and (ii) predation by Chaetognaths varies seasonally with changes in stage structure, not only in size structure (Feigenbaum, 1991 and references therein).

In the field, Irigoien et al. (Irigoien et al., 2004) observed that *Sagitta* spp. weighted mean depth (WMD) is well correlated in the Irish Sea to the *Pseudocalanus elongatus* and *Calanus* spp. WMD, but without gut content analysis these authors could not conclude that this was due to *Sagitta* spp. preference for these prey. However, chaetognaths have been shown to control copepod populations and standing stocks in different environments (i.e. Williams and Collins, 1985). They are ambush predators, sensing prey through vibrations (Feigenbaum and Maris, 1984) and are likely to be mainly size-selective predators (e.g. Pearre, 1980). Nevertheless, they also show selectivity between...
different copepod species (Rakusa-Suszczewski, 1969; Reeve and Walter, 1972; Pearre, 1973, 1974; Nagasawa and Marumo, 1976; Drits, 1981; Kimmerer, 1984; Baier and Purcell, 1997; Marazzo et al., 1997; Saito and Kiorboe, 2001).

Our molecular analysis of *S. setosa* gut contents shows that *C. helgolandicus* is present in the diet of *S. sagitta* throughout the year. Molecular analysis has unambiguously confirmed the presence of *Calanus helgolandicus* within the gut of *Sagitta setosa*. This work has shown that the DNA of consumed prey is not completely degraded during digestion by *Sagitta* and therefore can be amplified via PCR from the posterior two-thirds of the gut. However, the interpretation of negative amplifications must be treated with caution. A non-amplification could be a result of the particular organism not having being ingested, the material being too degraded for amplification, or because of a failed PCR reaction. Conversely, a positive amplification, particularly once sequenced, is an unmistakable proof of the organism studied having being ingested. This approach leads to very interesting results as *Calanus* has been rarely shown to be part of *S. setosa* diet (e.g. Rakusa-Suszczewski, 1969; Drits and Utkina, 1988).

We are aware that this approach is only qualitative and that the contribution of *C. helgolandicus* to the diet is likely to change during the year. When randomly picking up *S. setosa* in 95% ethanol for molecular analysis of gut content, we also counted how many of these transparent organisms contained one or several prey in their gut (Table II). Wimpenny (Wimpenny, 1937) in the south-west North Sea determined that the maximum proportion of *S. setosa* containing food was observed in May. In our study, the highest proportion of individuals with gut contents was observed from March (65%) to July (12%), then the rest of the year less than 10% of the organisms had material in their gut with very low percentages in winter.

Time of the day of sample collection might also be an important factor to consider. In our study, *S. setosa* were always collected at mid-morning. Wimpenny (Wimpenny, 1937) was the first to report that chaetognaths feed more actively at night than during the day when analysing the gut contents of *S. setosa* from the North Sea. Parry (Parry, 1944) also reported feeding
under low light conditions from laboratory observations. Therefore, it is likely that the organisms analysed in this study were not in the best context to have a full gut, meaning that the percentage of presence of Calanus helgolandicus in Sagitta gut content as well as our estimation of the predation impact on C. helgolandicus are minimal values.

Wimpenny's (Wimpenny, 1937) study in the south-west North Sea showed that patches of Calanus finmarchicus eggs and Sagitta setosa were generally coincident with or near diatom blooms which are considered in this area as breeding and nursery grounds (Savage and Wimpenny, 1936). McLaren (McLaren, 1969) observed that Sagitta elegans recruitment in Ogach Lake, Canada coincided with sharp increases in the abundance of Pseudocalanus nauplii, which would be expected to be greater with increased spring productivity. It is therefore not surprising that peaks of total egg production of Calanus helgolandicus coincide with the spring and autumn increase in Chl a at station L4 and are shortly followed by an increase in abundance of S. setosa (Fig. 7). McLaren (McLaren, 1969) concluded that timing in relation to food was more important than food level.

**Percentage of the Calanus helgolandicus population removed**

We estimated the minimum percentage of the Calanus helgolandicus population removed by Sagitta setosa daily. Highest predation pressures occur in summer (June: 6.3%, July: 18.9% and August: 8.8%), autumn (October: 8.9%) and winter (December: 6%) showing that Sagitta setosa can have a strong influence on Calanus helgolandicus population dynamics. Minimum predation pressure of S. setosa on C. helgolandicus ranges from 2.5% in February to 18.9% in July (Table II).

To our knowledge, there are only few estimates of chaetognath predation pressure on copepod populations in the field, as this approach needs a complete abundance and identification of the prey, an identification of chaetognath gut contents (Sameoto, 1972, 1973; Tönnesson and Tiselius, 2005) or a use of long-term series on prey–predator abundance (Clark et al., 2003; this paper). Duroé and Saiz (Duroé and Saiz, 2000) estimated a global impact of chaetognaths (seven congeneric species of Sagitta) on copepod standing stock ranging between 0.08 and 0.15% (based either on both copepods and adults, or only adults) in the Catalan Sea (Mediterranean). Similarly, Stuart and Verheye (Stuart and Verheye, 1991) estimated that between 1 and 5.3% of the copepod standing stock was consumed per day in the Benguela system. However, as first pointed out by Mironov (Mironov, 1960), predation impact on a prey should be compared with its productivity to really understand what is removed or replaced daily. For example, Sameoto (Sameoto, 1972, 1973) estimated that Sagitta elegans consumed between 0.07 and 1.1% of the...
annual production in St Margaret’s Bay and up to 36% in Bedford Basin (eastern Canada), making them the most important copepod predators there. However, these studies do not consider chaetognaths as selective feeders, as they estimate a predation pressure on the total copepod community. However, chaetognath species differ in the copepod prey found in their guts (Duró and Saiz, 2000; Tönnesson and Tiselius, 2005). Duró and Saiz (Duró and Saiz, 2000) found that Sagitta setosa showed a preference for prey about 70% of its own head width in the Mediterranean Sea. Thus, if the predation pressure is now calculated for targeted species of copepods, the impact could then be significant. For example, Duró and Saiz (Duró and Saiz, 2000) consider that chaetognaths could account for roughly 30% of Centropages productivity in the Mediterranean. To our knowledge, the only predation pressure estimates for S. setosa are from the recent study of Tönnesson and Tiselius (Tönnesson and Tiselius, 2005). They estimated that 26 to 48% of the Pseudocalanus population was removed daily when Sagitta setosa was abundant while its predation impact on Pterocalanus parucus ranged from 3 to 29% day$^{-1}$ and from 3 to 34% day$^{-1}$ on copepod nauplii. Our results show that the predation impact of S. setosa on the C. helgolandicus population can be rather important at station L4. However, as our study was not intended to be quantitative, we need to moderate these numbers and to take into consideration several points:

- we used a minimal number of prey per chaetognath (NPC = 1) when C. helgolandicus presence was positive in S. setosa guts as we did not have any information on the number of C. helgolandicus in the gut. In addition, we considered that the NPC was the same whatever the stage of development (or the size of the individual), as no data have been published on the feeding rates of S. setosa juveniles.
- Studies on S. setosa are less numerous than for its congeneric species S. elegans and Calanus helgolandicus has been rarely shown to be part of S. setosa diet (e.g. Rakusa-Suszczewski, 1969; Drits and Utikina, 1988).
- Therefore, we do not have any other estimations of predation pressure to compare with.
- If chaetognaths only are able to remove up to 19% of C. helgolandicus stock at some times of the year, the additional impact of other predators (e.g. siphonophores) will affect even more C. helgolandicus populations.

CONCLUSION

Calanus helgolandicus population dynamics is the result of the interaction of many parameters. Our long-term series and molecular biology approaches have shown that S. setosa is predating on C. helgolandicus. The minimal estimates of the impact of S. setosa on the C. helgolandicus population in our study range from 2.5 up to 19% suggesting that S. setosa is contributing to the structuring of C. helgolandicus population dynamics at station L4. However, there is an important need to estimate tropho-dynamic processes for chaetognaths and especially S. setosa under controlled conditions in the laboratory.

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