

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 and Utkina, 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 (Irigoin 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

Taxa	Species	Prey	NPC (prey ind. ⁻¹)	DT (h ⁻¹)	FR (prey day ⁻¹)	Specific daily ration (dry wt basis)	T (°C)	Location	Reference
Chaetognath	<i>S. setosa</i>		0.219		2.3		14	Gullmarsfjord, Sweden	Øresland (1987)
	<i>S. setosa</i>		0.250				6		
	<i>S. setosa</i>	Nauplii		2			11.5	Bay of Sevastopol	Mironov (1960)
				1.5			15.5		
			0.085 ^a	1	2.04		20		
					4.8 ^b	1.68–0.072 (larvae-adult)	20		
	<i>S. setosa</i>			5.5			15–20	Mediterranean Sea	Duró and Saiz (2000 and references therein)
	<i>S. setosa</i>			5				England	Parry (1944)
	<i>S. setosa</i>	Copepods, nauplii, chaetognaths, appendicularians	0.28 to 0.91	2.7 to 4.8			9 to 16	Gullmar fjord, Sweden	Tönnesson and Tiselius (2005)
	<i>S. setosa</i> + <i>S. elegans</i>	Copepods and fish larvae combined	0.245 ^a	2.88 ^a	2.04 ^b	0.062	15	Laboratory experiment	Kuhlmann (1976, 1977)
		Chaetognaths		4.16			15		
		Copepods		2.45			15		
		Fish larvae		0.9–5.2			15		

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): $FR = NPC \times 24/DT$, DT was estimated from temperature according to Ohman (1986): $DT = 10.48 e^{-0.086T}$, where T is the temperature in degree Celsius.

^bBased 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 (i.e. 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'-cgctgtttaacaaaacat-3'; Palumbi and Benzie, 1991) and the *Calanus* specific reverse primer 16SB2R (5'-attcaacatcgaggtcacaac-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*

	Jan 2005	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2006	Total
Number of <i>S. setosa</i> sorted	302	298	331	13	86	397	238	239	69	164	302	251	354	3044
Number of <i>S. setosa</i> with material in their gut	9	10	215	5	9	48	17	15	4	8	10	8	12	370
Number of <i>S. setosa</i> with material in their posterior 2/3 gut	9	9	212	5	8	47	16	14	4	8	9	7	10	358
Number of <i>S. setosa</i> with material in their posterior 2/3 gut analysed by PCR	3	3	3	3	3	3	3	3	3	3	3	3	3	39
Number of <i>S. setosa</i> from row above showing a positive PCR amplification for <i>Calanus</i>	1	2	3	1	2	2	1	2	0	1	0	0	2	17
FCR (% of <i>S. setosa</i> in the total sample with <i>C. helgolandicus</i> in their 2/3 posterior gut)	1.118	1.133	8.006	14.423	3.488	4.440	2.521	2.197	0.725	1.829	0.373	0.349	1.059	
Predation pressure on <i>C. helgolandicus</i> (%)	4.6	2.5	3.0	5.6	4.5	6.3	18.9	8.8	3.6	8.9	4.2	6.0	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 percentage of the total copepod abundance for those >1%: *Oncaea* spp. (26.9%), *Paracalanus parvus* (20.3%) (Tønnesson and Tiselius, 2005), *Pseudocalanus elongatus* (12.5%) (Tønnesson and Tiselius, 2005), *Temora longicornis* (7.8%) (Øresland, 1987), *Corycaeus anglicus* (2.6%) and *Euterpina 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): $\text{DT} = 10.48 e^{-0.086T}$, where T is the temperature in degree Celsius.

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

$$\text{PP} = I \times \text{FCR} \times \text{Nt} \times 100/\text{Nc}$$

with Nt, the total abundance of *Sagitta setosa* (ind. m⁻³), and Nc, 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

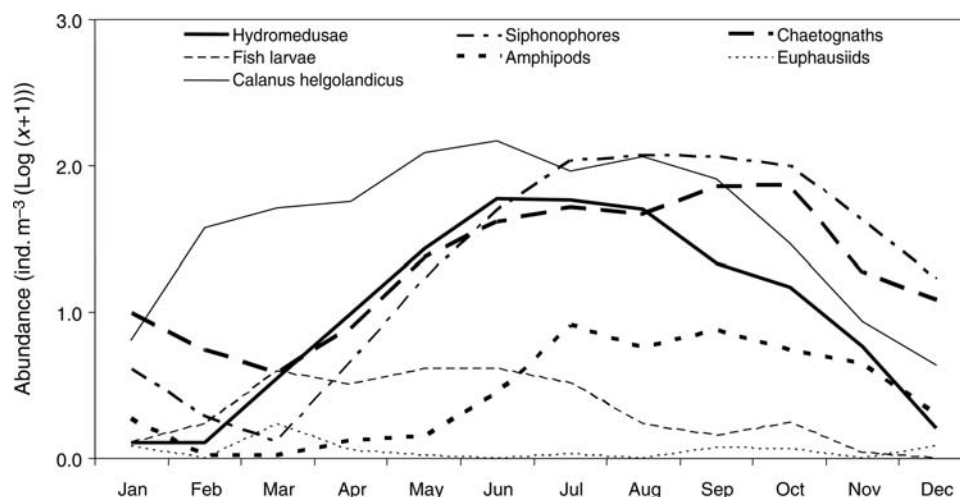


Fig. 1. Abundance (ind. m^{-3} ($\text{Log}(x+1)$)) of *Calanus helgolandicus* and its potential predators at station L4, Western English Channel.

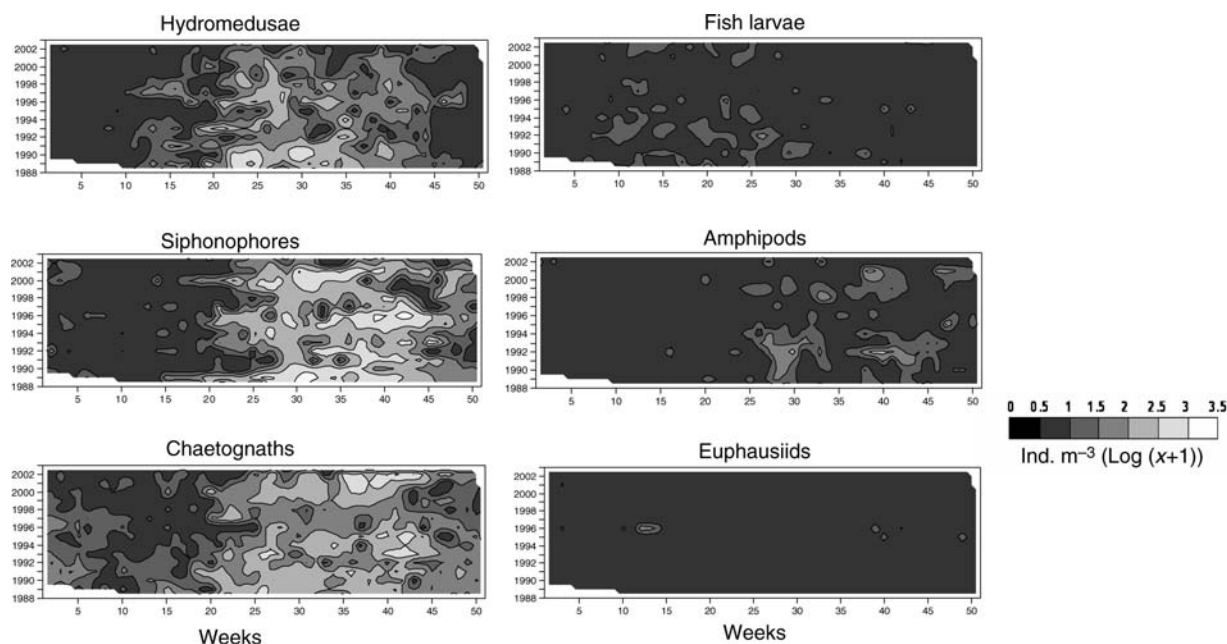


Fig. 2. Long-term abundance and seasonality of *Calanus helgolandicus* predators at station L4.

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 *C. 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

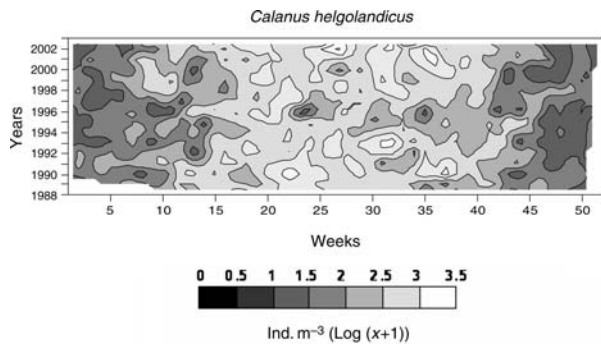


Fig. 3. Long-term abundance and seasonality of *Calanus helgolandicus* at station L4.

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 are 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

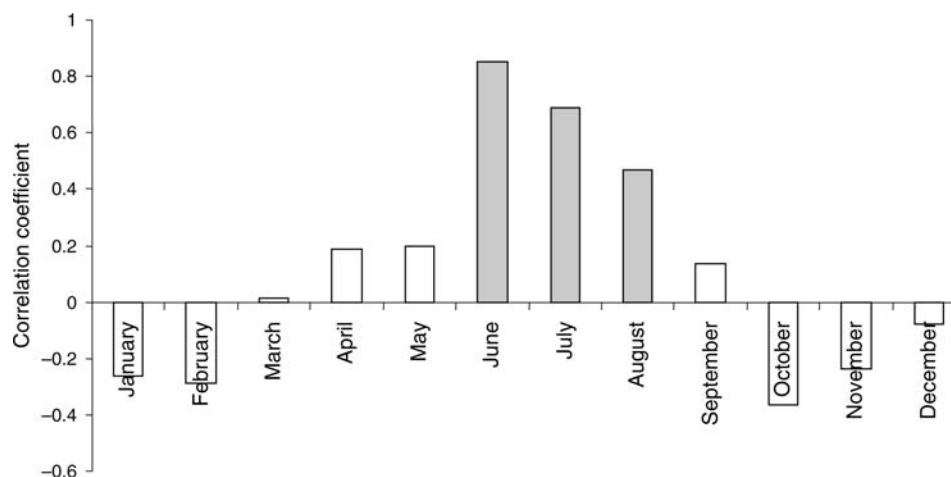


Fig. 4. Correlation coefficients calculated over the whole L4 time series between *Calanus helgolandicus* abundance for each month and its total maximum abundance during the year. Grey bars indicate significant correlations ($P \leq 0.05$).

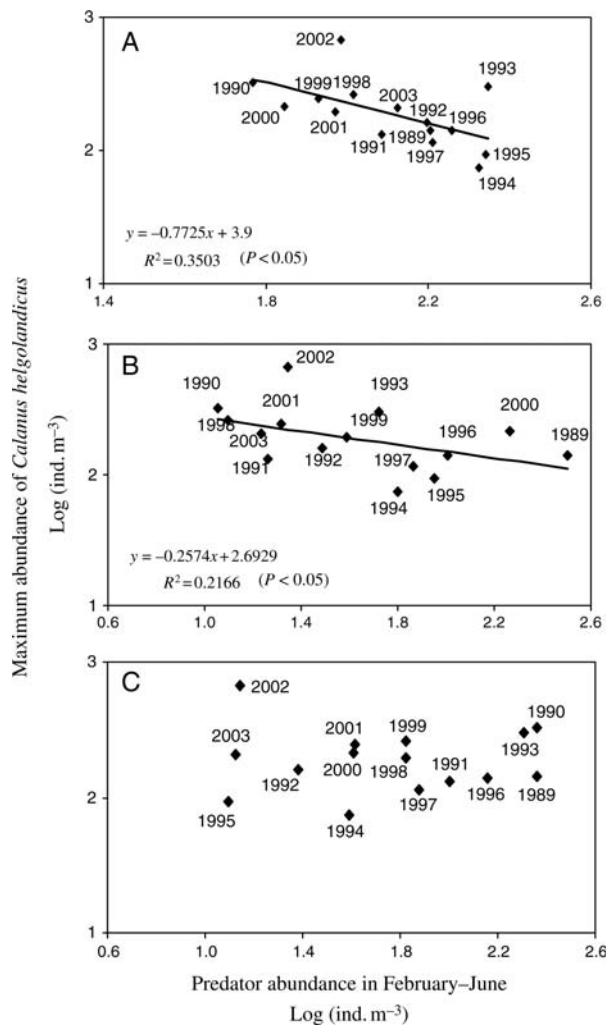


Fig. 5. Abundances of potential predators of *Calanus helgolandicus* from February to June in relation to *Calanus helgolandicus* maximum abundance throughout the time series. (A) Chaetognaths, (B) Siphonophores, (C) Hydromedusae. Significant inverse relationships are indicated.

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 Kjørboe, 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

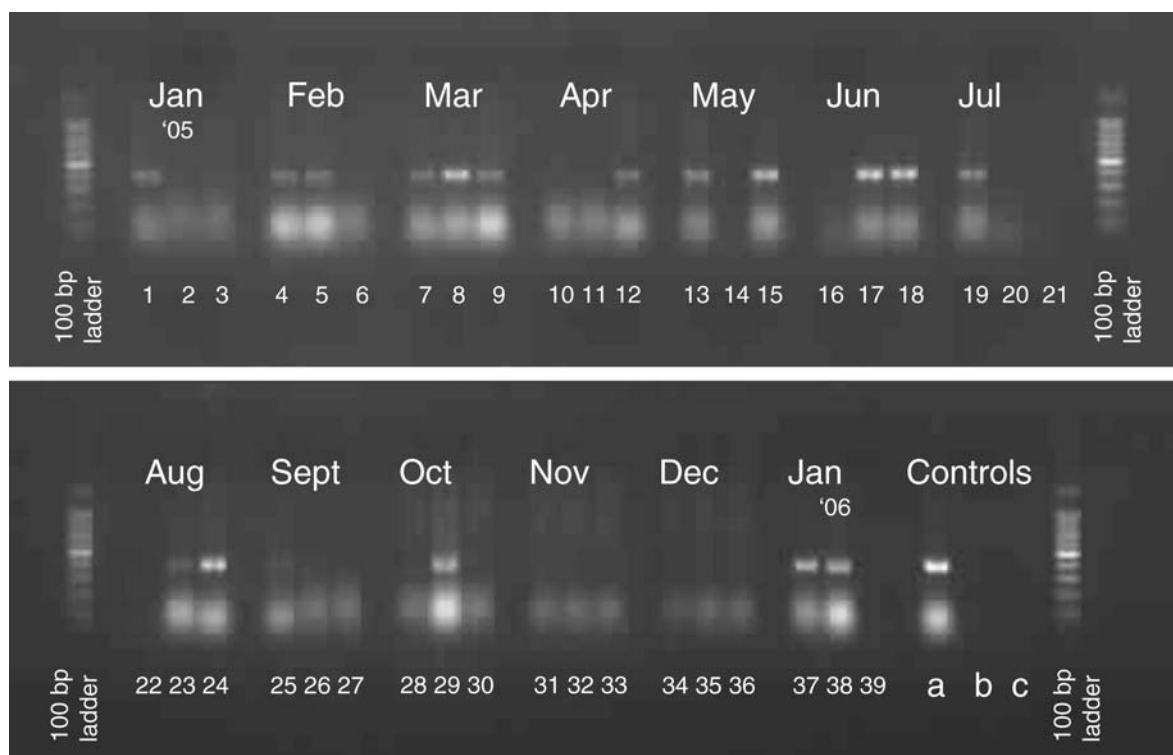


Fig. 6. Amplification of a region of 16S rDNA of *Calanus helgolandicus* from the gut content of *Sagitta setosa*. Individual amplifications are from: lanes 1, 2, 3—January 2005; 4, 5, 6—February; 7, 8, 9—March; 10, 11, 12—April; 13, 14, 15—May; 16, 17, 18—June; 19, 20, 21—July; 22, 23, 24—August; 25, 26, 27—September; 28, 29, 30—October; 31, 32, 33—November; 34, 35, 36—December; 37, 38, 39—January 2006. Seventeen of the 39 reactions were successful. Replicates for each reaction not shown. Control a, positive control of undigested identified *C. helgolandicus*; control b, no template control; control c, false positive control of a portion of *Sagitta setosa* containing no gut content. Either ends of the gels = 100 bp ladder DNA size-marker.

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 Kiørboe, 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 been 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 been 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

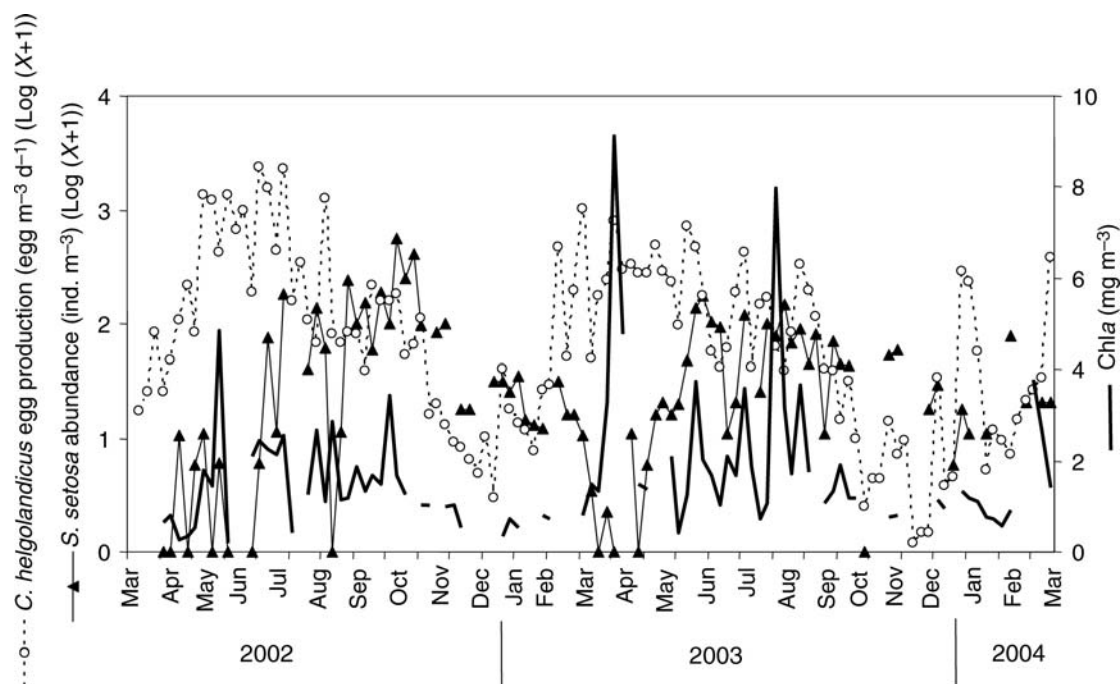


Fig. 7. Chlorophyll *a* (mg m^{-3}), total abundance of *Sagitta setosa* (ind. m^{-3}) ($\text{Log}(x+1)$) and *Calanus helgolandicus* egg production ($\text{egg m}^{-3} \text{ day}^{-1}$) ($\text{Log}(x+1)$) at station L4 between March 2002 and March 2004.

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 *C. 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 Ogac 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, 1973; Tönnesson and Tiselius, 2005) or a use of long-term series on prey–predator abundance (Clark *et al.*, 2003; this paper). Duró and Saiz (Duró 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 copepodites 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* sp. population was removed daily when *Sagitta setosa* was abundant while its predation impact on *Paracalanus parvus* 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 Utkina, 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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REFERENCES

- Álvarez-Cadena, J. N. (1992) Feeding habits, gonadic stages and size frequency distribution of *Sagitta setosa* J. Müller to the east of the Isle of Man, North Irish Sea. Port Marine Laboratory, Port Erin Isle of Man UK. *An. Inst. Cienc. Mar. Limnol.*, **19**, 215–222.
- Bacha, M. and Amara, R. (2009) Spatial, temporal and ontogenetic variation in diet of anchovy (*Engraulis encrasicolus*) on the Algerian coast (SW Mediterranean). *Estuarine Coastal Shelf Sci.*, **85**, 257–264.
- Bagoeien, E., Kaartvedt, S. and Oeveraes, S. (2000) Seasonal vertical migrations of *Calanus* spp. in Oslofjorden. *Sarsia*, **85**, 299–311.
- Bagoeien, E., Kaartvedt, S., Aksnes, D. L. et al. (2001) Vertical distribution and mortality of overwintering *Calanus*. *Limnol. Oceanogr.*, **46**, 1494–1510.
- Baier, C. and Purcell, J. E. (1997) Effects of sampling and preservation on apparent feeding by chaetognaths. *Mar. Ecol. Prog. Ser.*, **146**, 37–42.
- Bajkov, A. D. (1935) How to estimate the daily natural food consumption of fish under natural conditions. *Trans. Am. Fish. Soc.*, **65**, 288–289.
- Beaugrand, G., Reid, C., Ibanez, F. et al. (2002) Reorganisation of North Atlantic marine copepod biodiversity and climate. *Science*, **296**, 1692–1694.
- Blankenship, L. E. and Yayanos, A. A. (2005) Universal primers and PCR of gut contents to study marine invertebrate diets. *Mol. Ecol.*, **14**, 891–899.
- Bone, Q., Kapp, H. and Pierrot-Bults, A. C. (1991) *The Biology of Chaetognaths*. Oxford Science Publications, pp. 1–5.
- Bonnet, D., Titelmann, J. and Harris, R. (2004) *Calanus* the cannibal. *J. Plankton Res.*, **26**, 937–948.

- Clark, R. A., Frid, C. L. J. and Nicholas, K. R. (2003) Long-term, predation based control of a central-west North Sea zooplankton community. *ICES J. Mar. Sci.*, **60**, 187–197.
- Cushing, D. H. (1983) Are fish larvae too dilute to affect the density of their prey? *J. Plankton Res.*, **5**, 847–854.
- Davis, C. S. (1984) Predatory control of copepod seasonal cycles on Georges Bank. *Mar. Biol.*, **82**, 31–40.
- Drits, A. V. (1981) Some patterns of feeding of *Sagitta enflata*. *Oceanology*, **21**, 624–628.
- Drits, A. V. and Utkina, S. S. (1988) Feeding of *Sagitta setosa* in the layers of daytime phytoplankton accumulation in the Black Sea. *Oceanology*, **28**, 781–785.
- Durbin, E. G., Casas, M. C., Rynearson, T. A. *et al.* (2008) Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase I gene. *Mar. Biol.*, **153**, 699–707.
- Duró, A. and Saiz, E. (2000) Distribution and trophic ecology of chaetognaths in the western Mediterranean in relation to an inshore-offshore gradient. *J. Plankton Res.*, **22**, 339–361.
- Feigenbaum, D. L. (1991) Food and feeding behaviour. In Bone, Q., Kapp, H. and Pierrot-Bults, A. C. (eds), *The Biology of Chaetognaths*. Oxford University Press, New York, pp. 45–54.
- Feigenbaum, D. L. and Maris, R. C. (1984) Feeding in the chaetognaths. *Oceanogr. Mar. Biol., Annu. Rev.*, **22**, 343–392.
- Feigenbaum, D. and Reeve, M. R. (1977) Prey detection in the chaetognath: response to a vibrating probe and experimental determination of attack distance in large aquaria. *Limnol. Oceanogr.*, **22**, 1052–1058.
- Fortier, L., Le Fèvre, J. and Legendre, L. (1994) Export of biogenic carbon to fish and to the deep ocean: the role of large planktonic microphages. *J. Plankton Res.*, **16**, 809–839.
- Fraser, J. H. (1969) Experimental feeding of some Medusae and Chaetognaths. *J. Fish. Res. Board Can.*, **26**, 1743–1762.
- Frid, C. L. J., Newton, L. and Williams, J. A. (1994) The feeding rates of *Pleurobrachia* (Ctenophora) and *Sagitta* (Chaetognatha), with notes on the potential seasonal role of the planktonic predators in the dynamics of North Sea zooplankton communities. *Neth. J. Aquat. Ecol.*, **28**, 181–191.
- Gibbons, M. J. (1993) Diel vertical migration and feeding of *Sagitta friederici* and *Sagitta tasmanica* in the southern Benguela upwelling region, with a comment on the structure of the guild of primary carnivores. *Mar. Ecol. Prog. Ser.*, **111**, 225–240.
- Hirota, J. (1974) Quantitative natural history of *Pleurobrachia bachei* in La Jolla Bight. *Fish. Bull. US*, **72**, 295–335.
- Hirst, A., Bonnet, D. and Harris, R. P. (2007) Seasonal dynamics and mortality rates of *Calanus helgolandicus* over two years at a station in the English Channel. *Mar. Ecol. Prog. Ser.*, **340**, 189–205.
- Horridge, G. A. and Bolton, P. S. (1967) Prey detection in Chaetognaths via a vibration sense. *Proc. R. Soc. B*, **168**, 413–419.
- Hulselan, N. V. (1995) The temporal dynamics of three contrasting zooplankton communities with special reference to the role of zooplankton predators. *PhD Thesis*. University of Newcastle Upon Tyne.
- Irigoin, X. and Harris, R. P. (2003) Interannual variability of *Calanus helgolandicus* in the English Channel. *Fish. Oceanogr.*, **12**, 317–326.
- Irigoin, X., Conway, D. and Harris, R. P. (2004) Flexible diel vertical migration behaviour of zooplankton in the Irish Sea. *Mar. Ecol. Prog. Ser.*, **267**, 85–97.
- Kehayias, G. (2003) Quantitative aspects of feeding of chaetognaths in the eastern Mediterranean pelagic waters. *J. Mar. Biol. Assoc. UK*, **83**, 559–569.
- Kehayias, G., Michaloudi, E. and Koutrakis, E. (2005) Feeding and predation impact of chaetognaths in the north Aegean Sea (Strymonikos and Ierissos Gulfs). *J. Mar. Biol. Assoc. UK*, **85**, 1525–1532.
- Khulmann, D. (1976) *Experimentelle Untersuchungen zum Nahrungserwerb des Chaetognathen Sagitta setosa J. Müller und Sagitta elegans Verrill unter besonderer Berücksichtigung ihres Verhaltens gegenüber Fischbrut*. Diplom Arbeit University, Kiel, FRG.
- Khulmann, D. (1977) Laboratory studies on the feeding behaviour of the chaetognaths *Sagitta setosa* J. Müller and *S. elegans* Verrill with special reference to fish eggs and larvae as food organisms. *Meeresforsch.*, **25**, 163–171.
- Kimmerer, W. J. (1984) Selective predation and its impact on prey of *Sagitta enflata* (Chaetognatha). *Mar. Ecol. Prog. Ser.*, **15**, 55–62.
- Lindeque, P. K., Harris, R. P., Jones, M. B. *et al.* (1999) Simple molecular method to distinguish the identity of *Calanus* species (Copepoda: Calanoida) at any developmental stage. *Mar. Biol.*, **133**, 91–96.
- Marazzo, A., Machado, C. F. and Nogueira, C. S. R. (1997) Notes on feeding of Chaetognaths in Guanabara Bay, Brazil. *J. Plankton Res.*, **19**, 819–828.
- McLaren, I. A. (1969) Population and production ecology of zooplankton in Ogac Lake, a landlocked fiord on Baffin Island. *J. Fish. Res. Board Can.*, **26**, 1485–1559.
- Mironov, G. N. (1960) The food of plankton predators. 2. Food of *Sagitta*. *Trudy Sevastopol'skoi Biologicheskoi Stantsii*, **13**, 78–88.
- Nagasawa, S. and Marumo, R. (1976) Further studies on the feeding habits of *Sagitta crasa* Alvarinho in Suruga Bay, central Japan. *J. Oceanogr. Soc. Jpn.*, **32**, 209–218.
- Nejstgaard, J. C., Frischer, M. E., Raule, C. L. *et al.* (2003) Molecular detection of algal prey in copepod guts and fecal pellets. *Limnol. Oceanogr. Methods*, **1**, 29–38.
- Nejstgaard, J. C., Frischer, M. E., Simonelli, C. T. *et al.* (2008) Quantitative PCR to estimate copepod feeding. *Mar. Biol.*, **153**, 565–577.
- Newbury, T. K. (1972) Vibration perception by chaetognaths. *Nature*, **236**, 459–460.
- Ohman, M. D. (1986) Predator-limited population growth of the copepod *Pseudocalanus* sp. *J. Plankton Res.*, **8**, 673–713.
- Ohman, M. D. and Hsieh, C. H. (2008) Spatial differences in mortality of *Calanus pacificus* within the California Current System. *J. Plankton Res.*, **30**, 359–366.
- Oresland, V. (1987) Feeding of the chaetognaths *Sagitta elegans* and *Sagitta setosa* at different seasons in Gullmarsfjorden, Sweden. *Mar. Ecol. Prog. Ser.*, **39**, 69–79.
- Palumbi, S. R. and Benzie, J. (1991) Large mitochondrial DNA differences between morphological similar penaeid shrimp. *Mol. Mar. Biol. Biotechnol.*, **1**, 27–34.
- Parry, D. A. (1944) Structure and function of the gut in *Spadella cephaloptera* and *Sagitta setosa*. *J. Mar. Biol. Assoc. UK*, **26**, 16–36.
- Pearre, S. Jr (1973) Vertical migration and feeding in *Sagitta elegans* Verrill. *Ecology*, **54**, 300–314.
- Pearre, S. (1974) Ecological studies of three west-Mediterranean chaetognaths. *Inv. Pesq.*, **38**, 325–369.

- Pearre, S. (1980) Feeding by chaetognaths: the relation of prey size to predator size in several species. *Mar. Ecol. Prog. Ser.*, **3**, 125–134.
- Prahl, F. G., Eglinton, G., Corner, E. D. S. *et al.* (1985) Faecal lipids released by fish feeding on zooplankton. *J. Mar. Biol. Assoc. UK*, **65**, 547–560.
- Prokopchuk, I. (2009) Feeding of the Norwegian spring spawning herring *Clupea harengus* (Linne) at the different stages of its life cycle. *Deep Sea Res. II Top. Stud. Oceanogr.*, **56**, 2044–2053.
- Purcell, J. E. (1981) Dietary composition and diel feeding patterns of epipelagic siphonophores. *Mar. Biol.*, **65**, 83–90.
- Purcell, J. E. (1982) Feeding and growth of the siphonophore *Muggiaea atlantica* (Cunningham 1893). *J. Exp. Mar. Biol. Ecol.*, **62**, 39–54.
- Rakusa-Suszczewski, S. (1969) The food and the feeding habits of chaetognaths in the sea around the British Isles. *Pol. Arch. Hydrobiol.*, **16**, 213–232.
- Reeve, M. R. (1970) The biology of chaetognaths I. Quantitative aspects of growth and egg production in *Sagitta hispida*. In Steele, J. H. (ed.), *Marine Food Chains*. Oliver and Boyd, Edinburgh, pp. 168–189.
- Reeve, M. R. (1980) Comparative experimental studies on the feeding of chaetognaths and ctenophores. *J. Plankton Res.*, **2**, 381–393.
- Reeve, M. R. and Walter, M. A. (1972) Conditions of culture, food-size selection, and the effects of temperature and salinity on growth rate and generation time in *Sagitta hispida* Conant. *J. Exp. Mar. Biol. Ecol.*, **9**, 191–200.
- Reid, P. C., Edwards, M., Beaugrand, G. *et al.* (2003) Periodic changes in the zooplankton of the North Sea during the twentieth century linked to oceanic inflow. *Fish. Oceanogr.*, **12**, 260–269.
- Russell, F. S. (1951) A re-examination of *Calanus* collected off Plymouth. *J. Mar. Biol. Assoc. UK*, **30**, 313–314.
- Saito, H. and Kiørboe, T. (2001) Feeding rates in the chaetognath *Sagitta elegans*: effects of prey size, prey swimming behaviour and small-scale turbulence. *J. Plankton Res.*, **23**, 1385–1398.
- Sameoto, D. D. (1972) Yearly respiration rate and estimated energy budget for *Sagitta elegans*. *J. Fish. Res. Board Can.*, **29**, 987–996.
- Sameoto, D. D. (1973) Annual life cycle and production of the chaetognath *Sagitta elegans* in Bedford Basin, Nova Scotia. *J. Fish. Res. Board Can.*, **30**, 333–344.
- Savage, R. E. and Wimpenny, R. S. (1936) Phytoplankton and the Herring. Part. II. *Fish. Invest. Ser. II*, **15**, 1–88.
- Simonelli, P., Troedsson, C., Nejstgaard, J. C. *et al.* (2009) Evaluation of DNA extraction and handling procedures for PCR-based copepod feeding studies. *J. Plankton Res.*, **31**, 1465–1474.
- Sims, D. W. and Merrett, D. A. (1997) Determination of zooplankton characteristics in the presence of surface feeding basking sharks *Cetorhinus maximus*. *Mar. Ecol. Prog. Ser.*, **158**, 297–302.
- Stuart, V. and Verheye, H. M. (1991) Diel migration and feeding patterns of the chaetognath, *Sagitta friderici*, off the west coast of South Africa. *J. Mar. Res.*, **49**, 493–515.
- Sullivan, B. K. and Meise, C. J. (1996) Invertebrate predators of zooplankton on Georges Bank, 1977–1987. *Deep Sea Res. (II Top. Stud. Oceanogr.)*, **43**, 1503–1519.
- Symondson, W. O. C. (2002) Molecular identification of prey in predators diet. *Mol. Ecol.*, **11**, 627–641.
- Tremblay, J. E., Hattori, H., Michel, C. *et al.* (2006) Trophic structure and pathways of biogenic carbon flow in the eastern North Water Polynya. *Prog. Oceanogr.*, **71**, 402–425.
- Tønnesson, K. and Tiselius, P. (2005) Diet of chaetognaths *Sagitta setosa* and *S. elegans* in relation to prey abundance and vertical distribution. *Mar. Ecol. Prog. Ser.*, **289**, 177–190.
- Uye, S.-I., Yamaoka, T. and Fujisawa, T. (1992) Are tidal fronts good areas for herbivorous copepods? *Fish. Oceanogr.*, **1**, 216–226.
- Vestheim, H., Edvardsen, B. and Kaartvedt, S. (2005) Assessing feeding of a carnivorous copepod using species-specific PCR. *Mar. Biol.*, **147**, 381–385.
- Williams, R. and Collins, N. R. (1985) Chaetognaths and ctenophores in the holoplankton of the Bristol Channel. *Mar. Biol.*, **85**, 97–102.
- Wimpenny, R. S. (1937) The distribution, breeding and feeding of some important plankton organisms of the south-west North Sea in 1934. I. *Calanus finmarchicus* (Gunn), *Sagitta setosa* (J. Müller), and *Sagitta elegans* (Verrill). *Fish. Invest. London Ser. 2*, **15**, 1–53.
- Yen, J. (1985) Selective predation by the carnivorous marine copepod *Euchaeta elongata*: laboratory measurements of predation rates verified by field observations of temporal and spatial feeding patterns. *Limnol. Oceanogr.*, **30**, 577–597.