Comparison of zooplankton data collected by a continuous semi-automatic sampler (CALPS) and a traditional vertical ring net

SOPHIE G. PITOIS*, PAUL BOUCH, VERONIQUE CREACH AND JEROEN VAN DER KOOIJ

*CORRESPONDING AUTHOR: sophie.pitois@cefas.co.uk

Corresponding editor: Roger Harris

We compared and evaluated the performance of a Continuous Automatic Litter and Plankton Sampler (CALPS) against the traditional ring net vertical haul. CALPS is a custom-made semi-automatic sampler, which collects water using a pump system at a single depth along a predetermined transect as the ship sails. CALPS underestimated species abundance compared to the ring net by a factor 1.61, but both datasets illustrated a similar species composition, community size structure and good agreement in the spatial distribution of abundance. Our analysis suggests that avoidance of the CALPS is likely to be the main factor responsible for the observed difference in sampling efficiency, but other factors, such as depth, area sampled and zooplankton patchiness, are also likely to play their part. We conclude that whilst the CALPS is not suitable for investigations that require accurate measures of abundance, it is an ideal tool to identify and quantify changes in plankton communities and diversity. A particular advantage over more traditional vertical sampling methods is that it can be integrated within existing multidisciplinary surveys at little extra cost, thus making the CALPS particularly valuable as part of integrated monitoring programmes to underpin policy areas such as the EU Marine Strategy Framework Directive.

KEYWORDS: sampling efficiency; community structure; integrated monitoring; automated sampling

INTRODUCTION

In pelagic ecosystems, zooplankton occupies a central position in the food web, often controlling smaller organisms by grazing and providing food for many important larval and adult fish and seabirds (Pitois *et al.*, 2012; Lauria *et al.*, 2013). Zooplankton are also sensitive

indicators of climate variability as shown by studies in various regions (Brodeur and Ware, 1992; Edwards and Richardson, 2004; Hobday *et al.*, 2006; Richardson, 2008; Harrop and Edwards, 2014). As a result of the fundamental role played by zooplankton, considerable effort has been deployed in studying their abundance, distribution and changes through time. Zooplankton vary in size from the microscopic to large jellyfish and from robust to fragile and almost impossible to catch without damage. They also exhibit extremely diverse behaviours, daily and seasonal vertical migration, and different feeding, reproductive, survival and escape strategies. As a result, no single sampling device is able to sample all the zooplankton components at any one time and all systems underestimate at least parts of the zooplankton community, leading researcher to select the system they think is most appropriate to fulfil the aims of their particular studies (Batten *et al.*, 2013; Skjoldal *et al.*, 2013). As there are few dedicated monitoring sites and surveys, our knowledge of their biomass, size composition and rates of production in many shelf seas remains fragmentary; furthermore, zooplankton are difficult to simulate in ecosystem models and the lack of data hinders calibration of such models.

Resources for monitoring are always limited and outside the very few areas where dedicated zooplankton surveys are routinely conducted, such as the California Current (Bograd *et al.*, 2003) or the western Channel in the UK shelf (Smyth *et al.*, 2015) (see the COPEPOD project, http://www.st.nmfs.noaa.gov/copepod/ for a compilation of these survey results), it is desirable to develop cost-efficient methodologies and increase the time and space scales of sampling, by integrating zooplankton monitoring into multipurpose surveys (Shephard *et al.*, 2015). Such methodologies will need to combine cost effectiveness with scientific data quality sufficient to provide effective observational platforms for monitoring the planktonic ecosystem in relation to the environment and produce the necessary evidence base to support management decisions.

In the UK, efforts are underway to integrate plankton monitoring programmes (Scherer *et al.*, 2014). This is necessary because under Europe's Marine Framework Strategy Directive (MSFD—Directive 2008/56/EC establishing a framework for community action in the field of marine environmental policy), Member States are required to put in place the necessary management measures to achieve Good Environmental Status (GES) in their marine waters by 2020, and secondly establish and implement monitoring programmes to measure progress towards GES. According to Borja *et al.* (2013), GES is achieved if the integrity of food webs and the long-term abundance and reproduction of component species are maintained over time. For GES, zooplankton must be present and "occur at levels that are within acceptable ranges that will secure their long-term viability and functioning" and the "distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions".

The Continuous Automatic Litter and Plankton Sampler (CALPS) was developed with this in mind: it is a custom-made semi-automatic sampler which collects water using a pump system at a single depth and along a predetermined transect as the ship sails; the system can use up to six nets of different mesh sizes so as to be able to collect a wide range of size fractions of plankton and microplastic particles and fibres. A similar existing underway system is the Continuous Underway Fish Eggs Sampler (CUFES, Checkley *et al.*, 1997) that has been used worldwide to sample pelagic fish eggs [e.g. California Current (Weber *et al.*, 2015), Bay of Biscay (Albaina *et al.*, 2014), North Sea (Lelievre *et al.*, 2012)] and is also a good sampler for small zooplankton (Sono *et al.*, 2009). Underway systems such as the CUFES and CALPS operate continuously and under nearly all sea conditions, providing a real-time estimate of the volumetric abundance of particles at pump depth, and are thus particularly suitable for assessing aggregated distributions. The difference between CALPS and CUFES is that the CALPS can use a multinet system and sampling is automated.

In order to integrate data obtained with this new system with those obtained from other forms of sampling such as those used at fixed point where ring nets are deployed in a vertical haul, it is necessary to calibrate it against the more widely used gear used at the existing locations, in term of sampling efficiency and selectivity. This is because the two sampling systems use different methodologies: data from fixed point sampling sites and CALPS can all be used to monitor changes in the zooplankton, but they are likely to give different pictures of the plankton.

This paper considers the comparison, characterization and evaluation of the performance of the CALPS against the traditional and widely used method of vertical haul using a ring net. The aim of this study is:

(i) to compare the abundance and size of zooplankton collected from the CALPS with those collected with a ring net hauled from the seabed to the surface and examine whether the data collected by the CALPS reflect the vertically integrated abundances from the ring net;

(ii) to evaluate the routine use of the CALPS, as part of an integrated monitoring programme able to provide robust scientific data for the study of

planktonic ecosystems and able to provide the evidence base to support management decisions.

METHOD

Area of study and sampling strategy

The abundance and size of zooplankton collected from the CALPS were compared with those collected with a ring net hauled from the seabed to the surface during the PELTIC 2014 survey (PELagic ecosystem in the western English Channel and eastern CelTIC Sea). This was one of five integrated yearly monitoring surveys (2012–2016) conducted during the autumn (ICES, 2015). PELTIC 2014 was carried out from the 30th September to the 19th October on board the RV "Cefas Endeavour". Zooplankton samples were collected at 39 stations during night time (Fig. 1).

Sampling methodologies

Vertical hauls using ring net

Depth-integrated vertical hauls were made at the same 39 stations, from approximately 3 m above the seabed to the surface. An 80-µm-mesh net was used, mounted on a 0.5-m-diameter ring frame equipped with a General Oceanics mechanical flowmeter (model 2030RC, which includes a mechanism to prevent the rotor from turning backwards) mounted in the centre of the aperture of the net. A mini-CTD (SAIV) was attached to the bridle recording pressure (depth), temperature and salinity. The mesh size was chosen to reliably sample many of the smaller copepod species that are important grazers; it did not show any sign of clogging throughout the survey. The net was hauled to the surface at a speed of 0.5 m/s. This resulted in a volume filtered ranging from 3.6 to 67.2 m^3 per sample. The net was washed down and the end bag thoroughly rinsed with sea water before preserving the sample in 4% formaldehyde. Position, date, time, seabed depth and sampled depth (from CTD attached to net) were recorded and the volume filtered was calculated from the flowmeter readings.

CALPS

The CALPS consists of a pump system manufactured by 4H Jena Engineering GmbH and additional elements fitted onto the research vessel. The additional elements include a water inlet of 20 cm diameter, a flowmeter, six cylinder traps and associated valves and level detectors to prevent overflowing (Fig. 2). The CALPS is controlled by computer, so sampling start and finish can be programmed and triggered automatically at predetermined times and/or locations. When activated, the system pumps sea water from a depth of 4 m at rates of between 35 and 45 L/min and distributes the water into one or more of the six possible traps. Each

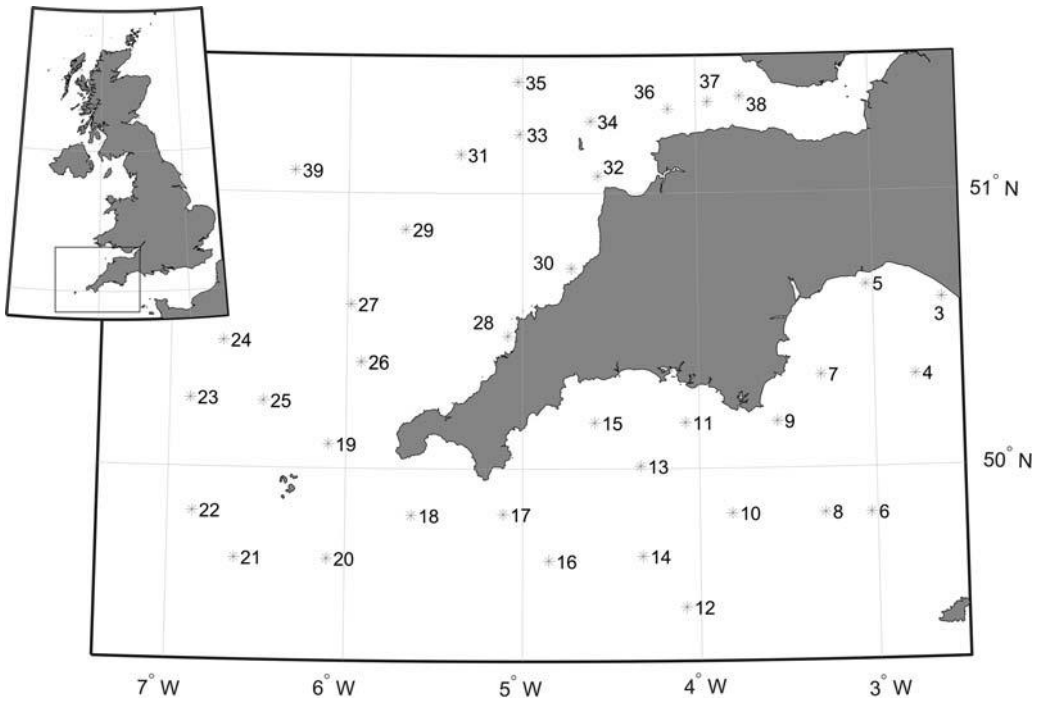

Fig. 1. Location of the 39 sampling locations where the ring net was deployed.

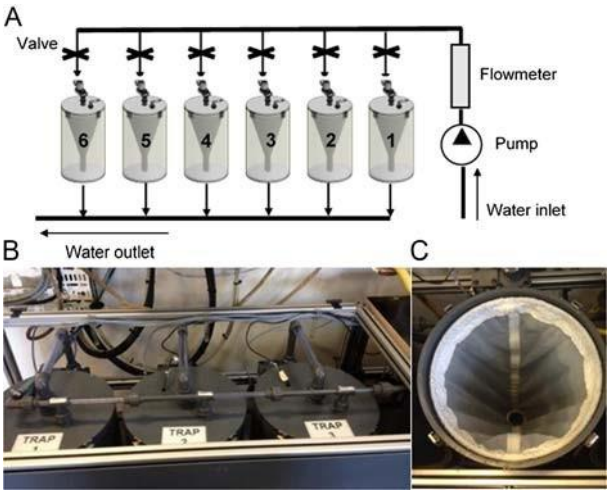

Fig. 2. (A) Schematic illustration of the CALPS system. (B) Photographs of the Traps system from above. (C) Plankton net inside each trap.

trap consists of a PVC cylinder (height: 73.3 cm, diameter: 28.0 cm) containing a plankton net (length 66.0 cm and diameter 26.5 cm) of chosen mesh size.

During the current survey, the samples were filtered through an 80-µm-mesh net, identical to that of the ring net. The volume of water filtered was measured with an electronic flowmeter, so that zooplankton abundance (m^{-3}) could be determined for each sample. Approximately 2000–2500 L water needed to be filtered to obtain a sufficiently large plankton sample for comparison with the ring net, corresponding to running the CALPS system for an hour. To achieve this without delaying vessel operations, sampling started while steaming at a fixed vessel speed of 10 knots, 20 min before arrival at the ring net station, continued during the deployment of the ring net at station (approximately 20 min), and was stopped 20 min after leaving the station at 10 knots vessel speed. The starting time and position, as well as end time, position and volume filtered were recorded for each station, the latter ranging from 1.9 to 3.9 m^3 of seawater filtered per sample.

Analysis of samples

Samples were analysed using the Zooscan Imaging system (Hydroptic v2.0). The samples preserved in 4% formaldehyde solution were first rinsed with deionized water. When high densities of zooplankton were present, sub-sampling was applied using a Folsom splitter, with the aim to include between 800 and 1200 objects, thus maximizing sample size while reducing the risk of specimens overlapping. The sub-sample was then poured into the scanning cell and overlapping objects were separated using needles. The scanned image was processed using the Zooprocess and Plankton Identifier software (Grosjean *et al.*, 2004; Gorsky *et al.*, 2010). A learning set based on a subset of vignettes from plankton samples collected during the current and previous years' surveys was used to automatically categorize the specimens into different taxonomic groups. Finally, an expert taxonomist manually validated the classifications. A series of metrics including size were automatically exported.

A total of 33 taxonomic groupings were identified in the samples. Calanoid and cyclopoid copepods were identified as far as possible to genus level. The exception was the *Para-pseudocalanus* taxonomic group, which also included all species of *Paracalanus*, *Pseudocalanus*, *Ctenocalanus*, *Clausocalanus* and *Microcalanus*. These genera could not consistently be distinguished and separated from the vignettes.

Numerical analysis

Abundance values (numbers per m^3) were transformed ($\log_{10}(x + 1)$) to reduce the asymmetry of the data. To compare abundances between the ring net and CALPS datasets, the transformed abundances of the dominant taxa (i.e. those contributing to at least 1% of the total zooplankton abundance) and total zooplankton, at each sampling location, were plotted and compared visually. To enable a taxon-by-taxon comparison of the abundances, the ratio between the CALPS and the ring net abundances (RingNet:CALPS) for these dominant taxa was calculated for each station with positive abundances for both datasets. An overall mean ratio was also calculated with associated standard deviation. To compare the raw, non-normally distributed abundance values from both gears at each station, the non-parametric Wilcoxon Signed Rank test was used (Wilcoxon, 1945). Correlation coefficients were calculated on $\log_{10}(x + 1)$ abundance data to determine which taxa were displaying good synchrony across the 39 sampling locations.

Bray–Curtis similarity coefficients between individual sample estimates of $\log_{10}(x + 1)$ transformed species abundance and species composition (proportion contributed by each taxon to total abundance) were calculated using the PRIMER-7 software (Plymouth Routines In Multivariate Ecological Research, Clarke and Warwick, 1994). Analyses of similarities (ANOSIM) were performed to test for differences between all ring net and CALPS samples with respect to species abundance and composition, and multi-dimensional scaling (MDS) plots were produced for the species composition similarity matrices.

Mean sizes of zooplankton, and associated standard deviations, were calculated for each taxonomic group and each sampling device, across all species analysed in

samples and all stations. One-way Analysis of Variance (ANOVA) was used to test for the effect of sampling gear on the mean size of the individual organisms caught for each taxon.

RESULTS

The most abundant taxa recorded from the CALPS were, in decreasing order, *Para/pseudocalanus* spp., unidentified copepods, *Acartia* spp., *Oithona* spp., bivalve larvae, *Corycaeus* spp., harpacticoid copepods, *Centropages* spp., gastropod larvae and copepod nauplii, altogether representing 95.30% of the total abundance. The most abundant taxa recorded from ring net sampling were, in decreasing order, *Para/pseudocalanus* spp., unidentified copepods, *Oithona* spp., *Acartia* spp., harpacticoid copepods, *Corycaeus* spp., bivalve larvae, chaetognatha, *Centropages* spp., appendicularia, *Calanus* spp., polychaete larvae, copepod nauplii and gastropod larvae, altogether representing 97.44% of the total zooplankton abundance (see Table S1 in Supplementary material for full details). These groups contributed to at least 1% of the total zooplankton abundance recorded with each device and were common in both datasets, apart from chaetognatha, appendicularia, *Calanus* spp. and polychaete larvae which contributed to >1% of the total abundance in the ring net dataset only. Only the first two taxonomic groups (*Para/pseudocalanus* spp. and unidentified copepods) were ranked in the same order. As the rank positions increased so did the discrepancies between the two datasets.

Comparison of zooplankton abundances

Differences in abundance were apparent between the CALPS and the ring net (Fig. 3 and see Fig. S1a,b in Supplementary material for species-specific plots). In most cases, the total zooplankton abundance estimated from ring net samples was higher than that estimated from CALPS samples (Fig. 3); out of 39, only 13 stations showed higher total zooplankton abundance recorded by the CALPS. The higher ring net abundances were mostly due to large differences recorded for *Oithona* spp., harpacticoid copepods, chaetognatha, *Calanus* spp., polychaete larvae and appendicularia. In particular, appendicularia were captured in one CALPS sample only while being present in most ring net samples. Only two taxonomic groups, cnidaria and unidentified cyclopoids, showed abundances recorded by the CALPS sampler that were more than twice higher than those recorded by the ring net. These two taxa however were minor contributors (i.e. <1%) to total zooplankton abundance. A one-way ANOSIM analysis showed that although

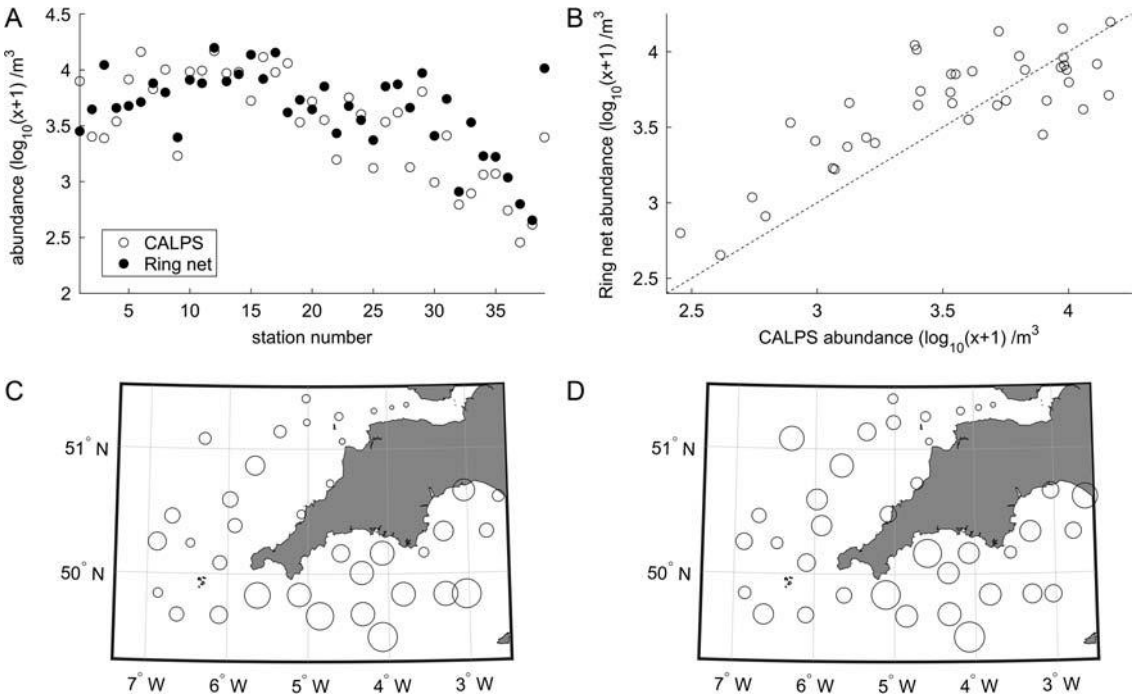

Fig. 3. (A) Total zooplankton abundance (individuals m^{-3}, with $\log_{10}$ (x + 1) transformation) from CALPS and ring net devices at the 39 sampling locations, R = 0.63, P < 0.001; (B) ring net (vs) CALPS total zooplankton abundance with dashed line representing Ring net = CALPS; and distribution of total zooplankton collected with the CALPS (C) and ring net (D), at the 39 sampling locations plotted using the same scale.

sample similarities between individual taxa abundance from the CALPS and ring net groups were different to sample similarities within groups, these difference were small and within the 90% confidence interval (R = 0.172, P = 0.1).

On average, the total zooplankton abundances recorded from the ring net were 1.61 higher than those recorded from the CALPS. Ratios of abundance (RingNet:CALPS) calculated for the dominant taxa varied between 1.30 for *Centropages* spp. and 39.3 for appendicularia (Table I). However, the latter was based on only one station only where appendicularia were captured by both sampling gears and is thus not reliable. Although abundances were variable between the two datasets, analysis of paired zooplankton counts obtained from the two devices revealed significant differences (Wilcoxon test: $P < 0.05$) only for *Oithona* spp., bivalve larvae, harpacticoids, chaetognatha, appendicularia and *Calanus spp*. Moreover, correlation coefficients above 0.5 indicate that relationships exist between the variability of zooplankton recorded by the CALPS and ring net sampling devices; half of the taxonomic groups in Table I show significant positive relationships and no significant difference between the datasets from both devices (Table I, see also Fig. S1 in Supplementary material for species-specific plots).

To test whether water column depth affected the sample size and the abundance of the organisms collected by each device, we looked at the relationships between depth sampled and volume filtered as well as with species-specific RingNet:CALPS ratios (Fig. 4). Pearson's correlations were also calculated; no significant relationship was found between depth sampled and RingNet:CALPS ratio for total zooplankton abundance (R = 0.30, P = 0.302), and a weak but significantly positive relationship was found between depth sampled and volume filtered (R = 0.564, $P < 0.001$).

Comparison of zooplankton community structure

The MDS analysis performed on the similarity matrices of relative abundances and associated plot (Fig. 5) showed no obvious separation of similarity coefficients. A one-way ANOSIM analysis (Global R = 0.111, P = 0.001) showed that, on average, similarities between groups and within groups were similar. This suggests that although differences in absolute zooplankton abundances were noticeable between the two datasets, the taxonomic groups captured by each device were similar.

Comparison of zooplankton sizes

Mean sizes calculated for each taxonomic group were generally higher from individuals caught in the ring net than in the CALPS (Table II, Fig. 6). The largest differences were for cnidaria, decapod larvae and appendicularia which were on average at least 80% larger in samples collected using the ring net. However, appendicularia were only recorded in one CALPS sample and this

Table I: Comparison of the abundances of the main zooplankton taxa collected from the CALPS and ring net devices

Taxa	Ratio of abundances (RingNet:CALPS)	Number of points	Correlation coefficient across 39 stations	Wilcoxon Test *P*-value
Para-pseudo calanus spp.	1.39 ± 0.94	38	0.9231 (<0.001)	0.971
Acartia spp.	3.52 ± 1.91	33	0.6553 (<0.001)	0.365
Oithona spp.	3.45 ± 2.41	37	0.7763 (<0.001)	<0.001
Bivalve larvae	1.48 ± 2.17	35	0.4165 (0.008)	0.016
Corycaeus spp.	1.60 ± 1.70	35	0.7620 (<0.001)	0.922
Harpacticoid copepods	2.58 ± 2.76	36	0.5229 (<0.001)	0.006
Centropages spp.	1.30 ± 1.23	35	0.6921 (<0.001)	0.202
Gastropod larvae	1.69 ± 2.37	29	0.2174 (0.1837)	0.094
Copepod nauplii	2.68 ± 3.87	29	0.5527 (<0.001)	0.207
Chaetognatha	6.16 ± 5.62	31	0.3537 (0.027)	<0.001
Appendicularia	39.30	1	0.1650 (0.316)	<0.001
Calanus spp.	2.35 ± 1.91	22	0.5259 (<0.001)	<0.001
Polychaete larvae	7.93 ± 11.63	6	0.6847 (<0.001)	n/a
Total zooplankton	1.61 ± 1.04	39	0.7953 (<0.001)	0.151

Column 2: Ratio of abundances RingNet:CALPS ± 1 Standard Deviation; Column 3: number of sampling locations with positive abundances recorded from both devices; Column 4: Pearson's correlation coefficients between $\log_{10}(x + 1)$ abundances resulting from both sampling devices calculated at all 39 sampling locations, R (P-value). Those positive and significant relationships with $R > 0.5$ and $P < 0.05$ are shown in bold; Column 5: P-value resulting from the Wilcoxon Signed Rank test on raw abundance values recorded from both devices. A P-value > 0.05 (emboldened) indicates that there is no significant difference in the series recorded by the two devices (i.e. the median difference of the distributions is close to zero). The taxonomic groups for which correlations are indicated and no significant difference between the datasets from both devices are greyed out.

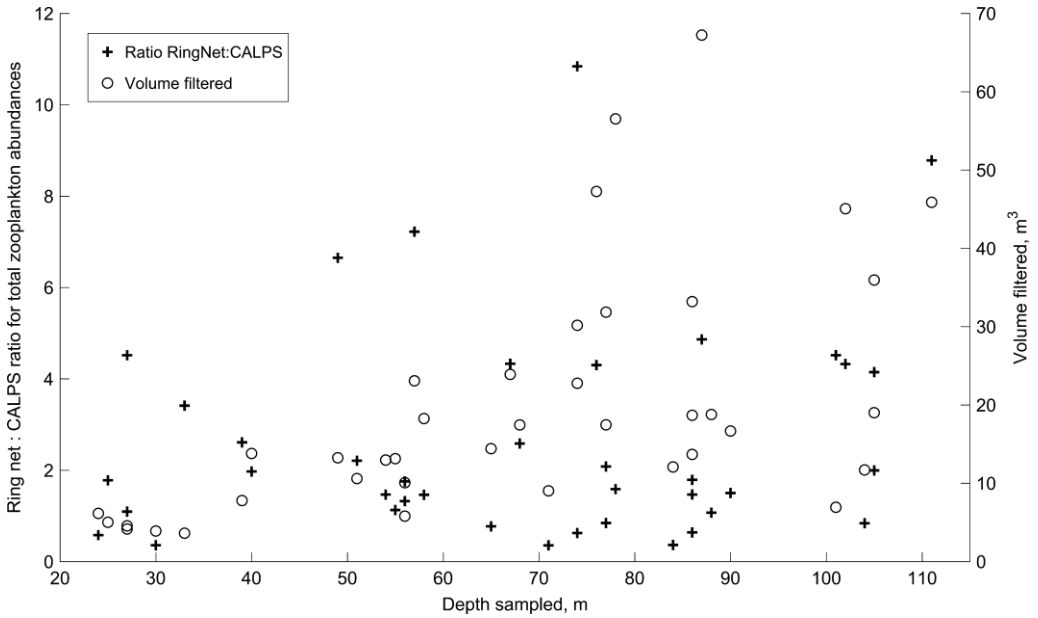

Fig. 4. Ratio RingNet:CALPS for the total abundance of zooplankton and volume filtered by the ring net as a function of depth of the water column sampled for each of the 39 data points.

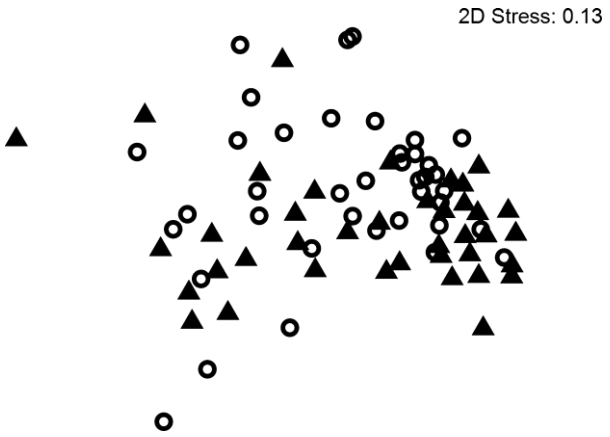

Fig. 5. Relative abundances contributed by individual taxa to total abundance from samples taken with the CALPS (black triangles) and ring net (open circles). Non-metric MDS scatter plot of all samples collected with the CALPS and ring net based on the rank order of sample similarities.

result is therefore statistically doubtful. Mean sizes for gammarids, polychaete larvae, *Calanus* spp., *Temora* spp. and *Centropages* spp. groups were also bigger in ring net samples, differences ranging from 36.17% from gammarids to 24.09% for *Centropages* spp. But again, these differences were based on only four samples for gammarids. Results from the one-way ANOVA test ($P < 0.05$) also showed differences for cnidaria, decapod larvae, appendicularia, *Calanus* spp., *Centropages* spp. and *Temora* spp., but not for the polychaetes and gammarids groups; this was due to a wider range of sizes recorded in the ring net samples, and the low number of positive gammarids records in CALPS samples. The *Para-pseudocalanus* groups show little differences in mean size between the two gears (6.22%), but the one-way ANOVA show statistical differences as a result of very narrow spread of the sizes of the organisms recorded by each gear.

DISCUSSION

Both the CALPS and ring net datasets illustrated a similar zooplankton community, with *Para-pseudocalanus* spp., *Acartia* spp. and *Oithona* spp., representing the most abundant taxa sampled by both devices. The spatial distribution of the total zooplankton abundance estimated with the two sampling methods was also similar (Fig. 3), and there was good agreement in abundance series recorded by the two devices for most individual taxa (Table I and Fig. S1 in Supplementary material). However, the abundance and rank of the taxa sampled differed from one dataset to the other, and although abundances were on average 1.61 times higher in samples collected from the ring net compared to those from the CALPS, individual RingNet:CALPS ratios varied between 1.30 and 39.3. The highest ratio was observed for the appendicularia, and it is clear that the CALPS' efficiency at capturing these organisms is very poor, seeing they were recorded at most stations when using the ring net, with an average density of 111.87 individuals m^{-3}. It is possible that these

Table II: Comparison of mean sizes (total length) of the zooplankton taxa collected from the CALPS and ring net devices

Taxa	CALPS, mean length (mm) ± std	Number positive samples	Ring net, mean length (mm) ± std	Number positive samples	Mean size difference ring net vs CALPS (%)	ANOVA *F*-ratio (*P*)
Para-pseudo calanus spp.	0.774 ± 0.063	38	0.822 ± 0.069	38	+6.22	8.661 (0.004)
Acartia spp.	0.945 ± 0.095	34	1.033 ± 0.282	37	+9.34	0.147 (0.706)
Oithona spp.	0.562 ± 0.049	38	0.559 ± 0.037	38	−0.56	0.231 (0.632)
Bivalve larvae	0.380 ± 0.022	37	0.397 ± 0.050	37	+4.40	2.151 (0.147)
Corycaeus spp.	0.799 ± 0.115	36	0.827 ± 0.119	37	+3.57	0.746 (0.391)
Harpacticoid copepods	0.610 ± 0.087	38	0.595 ± 0.037	37	−2.47	1.355 (0.248)
Centropages spp.	1.086 ± 0.207	36	1.347 ± 0.334	35	+24.09	6.894 (0.011)
Gastropod larvae	0.427 ± 0.054	34	0.433 ± 0.072	33	+1.52	0.044 (0.835)
Copepod nauplii	0.430 ± 0.048	32	0.431 ± 0.063	32	+0.12	0.036 (0.851)
Chaetognatha	2.785 ± 2.017	34	2.699 ± 2.138	35	−3.09	0.065 (0.800)
Appendicularia	0.628	1	1.171 ± 0.306	32	+86.39	5.194 (0.030)
Calanus spp.	1.526 ± 0.401	23	1.975 ± 0.521	32	+29.43	9.436 (0.003)
Polychaete larvae	0.591 ± 0.179	33	0.765 ± 0.435	10	+29.48	0.851 (0.372)
Temora spp.	0.873 ± 0.170	21	1.094 ± 0.306	24	+25.39	7.789 (0.008)
Cnidaria	0.785 ± 0.157	12	1.607 ± 1.211	10	+104.89	4.693 (0.042)
Decapod larvae	1.846 ± 0.912	19	3.504 ± 1.723	22	+89.77	11.69 (0.002)
Bryozoa	0.681 ± 0.151	20	0.642 ± 0.147	14	−5.68	0.550 (0.464)
Oncaea spp.	0.565 ± 0.054	11	0.546 ± 0.068	18	−3.29	0.965 (0.335)
Echinoderm larvae	0.496 ± 0.050	9	0.524 ± 0.115	10	+5.65	0.433 (0.520)
Unidentified cyclopoids	0.620 ± 0.073	15	0.599 ± 0.061	2	−3.45	0.157 (0.698)
Gammaridae	1.234 ± 0.218	4	1.680 ± 0.752	11	+36.17	0.723 (0.409)

Columns 2 and 4: Mean total lengths and associated standard deviation calculated from all individuals counted across the 39 samples from both CALPS and ring net devices. Columns 3 and 5: The number of positive samples is the number of sample where presence of a specific taxon was recorded. Column 6: Relative difference in mean size difference (%) calculated from differences between average values from samples obtained with CALPS and ring net datasets; (+) indicate that individuals in ring net samples are larger than those in CALPS. Column 7: Results of ANOVA (*F* ratio (*P*-value)) on the effect of sampling gear on the mean size of the individual organisms caught for each taxon; a high *F*-ratio with a low *P*-value (<0.05) indicates significant differences in the sizes of the organisms as a result of sampling gear used.

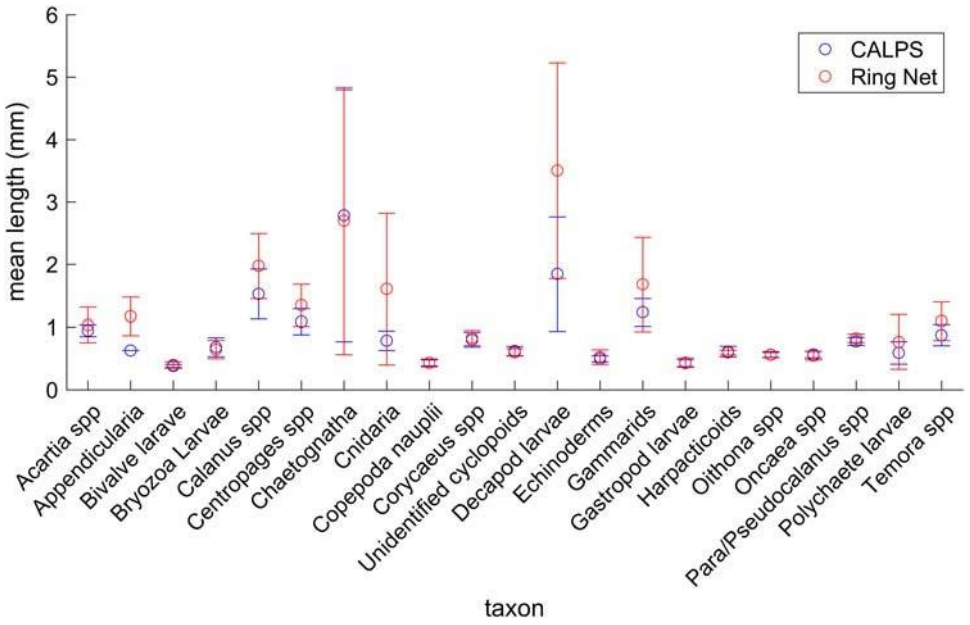

Fig. 6. Mean size (total length) of zooplankton and associated standard deviations calculated across all individuals analysed in samples from the 39 stations and for all taxonomic groups representing at least 0.1% of the total zooplankton abundance.

fragile organisms were damaged beyond recognition, as this has been reported in previous studies comparing pump systems with ring net deployment (Møhlenberg, 1987). For the polychaetes and chaetognatha, the RingNet:CALPS ratios were 7.93 and 6.16 respectively, indicating that these groups were also poorly captured by the CALPS. Dixon and Robertson (1986) also found significantly greater numbers of chaetognaths, polychaete

larvae and appendicularia in plankton nets rather than their pump system. They concluded that swimmers such as the chaetognaths probably have greater ability to avoid the pump intake, and that the much greater volumes of water sampled with the net were also a contributory factor. This suggests that the zooplankton community was sampled with different degrees of efficiency as is indeed confirmed by the results of the multivariate analyses. The most apparent difference between the two datasets is the generally higher abundances recorded by the ring net. Both gears were deployed at the same time and location, they both used an identical mesh size and the analysis of the samples was done in a standard way using Zooscan. Several factors were identified that could be responsible for the observed dissimilarity. These include differences in spatial area sampled, depth sampled, volume filtered, sampler and associated sampling design. Attributes such as avoidance behaviour of the organisms could also have contributed. We will discuss each of these points separately.

Spatial coverage

A perhaps obvious reason for potential discrepancies in zooplankton quantities sampled by the two devices is the differences in spatial coverage; the CALPS system was deployed for an hour, two-thirds of which while steaming at 10 knots. This meant that sampling was conducted over approximately 7 nautical miles compared to a single (stationary) point of the ring net. Zooplankton is inherently patchy (Mackas *et al.*, 1985) and the long horizontal sampling of the CALPS will integrate patches of high zooplankton abundance over large areas with lower densities, resulting in average abundances estimated across the area sampled. If this significantly contributed to the observed differences, we would also have expected to find stations where the abundances in the ring net were lower than those from the CALPS, i.e. when the sample station was situated in an area of low plankton abundance. This occurred only at 13 stations out of 39 (Fig. 3), and it is unlikely that this factor alone can be responsible for the differences in abundance between the two datasets. Our approach was consistent across stations; however, using underway sampling at different ship speeds results in spatially different sampling effort and integration of the sampling over different distances. We do not believe this to affect substantially the composition of the zooplankton community, but in order to avoid any such effect in routine deployments, CALPS should be used at a constant ship speed.

Depth sampled

Another clear difference between the two sampling methods was that the CALPS collected water at a fixed depth of 4 m below the surface, whereas the ring net sampled the entire water column. No stratification was recorded from CTD casts, but we aimed to reduce the effect of zooplankton vertical distribution in the water column to this sampling offset, by collecting all zooplankton samples at night time, when zooplankton tend to rise towards the surface (Lampert, 1989). The CALPS was therefore expected to be more effective than the ring net at sampling most zooplankton species apart from those that inhabit demersal habitats. This effect could have been mitigated by vertical mixing of the water column from turbulence and water displacement resulting from the passage of the ship. We also expected this water mixing of the surface layers to remove or minimize any potential sub-surface peak of zooplankton abundance. Previous studies comparing vertically integrated versus surface sampling methods concluded that differences in sampling depth could not be responsible for much the observed differences in abundance between the two sampling methodologies (Clark *et al.*, 2001; Richardson *et al.*, 2004). In our study, the lack of any relationship between RingNet:CALPS ratios and depth ($R = 0.17$, $P = 0.30$, Fig. 4) suggests that "sampled depth" may influence the sampling efficiency of the device but we do not expect this to be substantial.

Volume ftltered

As the station depth sampled increased, the volumes of water filtered by the ring net (Fig. 4) generally became much higher than those by the CALPS which consistently filtered 2–2.5 m^3. A positive weak but significant relationship was seen between volume filtered by the ring net and depth sampled ($R = 0.564$, $P < 0.001$). However, the volume filtered is also influenced by currents as a result of tide or high winds, which tend to pull the net frame away from the ship as it gets lowered; this effect can be substantial in strong currents and the further away the nets are taken from the ship, the higher the volume filtered. This can explain the high variability of volume filtered at the deeper station. Also, anecdotal reports have suggested that, despite the presence of a ball-bearing clutch, the model of flowmeter used here may, at times, rotate backwards when operated in vertical mode. Careful visual inspection of the flowmeter during deployment ensured that this was not likely, however, we cannot completely rule out the possibility that some volume readings may have been adversely affected by this.

Sampler and associated sampling design

Extrusion through the net mesh

Extrusion of animals through the net mesh is dependent on the mesh size and the tow speed. Faster towing speed increases filtration pressure on the mesh and consequently increases escapement of the smaller organisms by extrusion (Tranter and Smith, 1968). The effect of towing speed on the extrusion of smaller organisms through the net can be substantial and adds to the loss of organisms due to escapement (Skjoldal *et al.*, 2013). In the current study, the ring net generally filtered much higher quantities of water over a much shorter period (i.e. 3.6–67 m^3 taking a maximum of a few minutes) than the CALPS (1.9–3.9 m^3 over a period of 40 min to 1 h), and this would suggest a higher filtration pressure on the mesh and associated extrusion for the ring net. Although taxon-specific average lengths were generally higher in ring net samples (Fig. 6), only a few species showed statistically significant differences (i.e. cnidaria, decapod larvae, *Calanus* spp., *Temora* spp., *Centropages* spp. and *Para-pseudocalanus* spp., Table II); some of these taxa were the largest caught during this survey. If extrusion was involved, we would expect: firstly this effect to be highest for the smaller taxonomic groups, resulting in ratios of abundance RingNet:CALPS <1 for these groups; secondly a truncation towards the lower end of size spectra in ring net compared to CALPS samples, consistently across taxa; and thirdly organisms caught in ring nets to be in poorer condition than those in CALPS samples. None of these effects were observed (Fig. 6, Tables I and II). Differences in filtration efficiency for the two devices are therefore unlikely to explain the higher abundances recorded by the ring net.

Active and passive avoidance to the sampler

Sampling efficiency depends on factors such as towing speed, net mouth diameter and sampler and method design. It can result not only in a general underestimation of abundance but also in selective sampling. This avoidance can be passive or active. Passive avoidance results from particles being pushed away from the sampler mouth. As the aperture for the water inlet for the CALPS is smaller (i.e. 20 cm diameter) than the ring net (i.e. 50 cm diameter), it is suggested that the hydrodynamic effects produced by the CALPS sampler and associated with the ship's movement will be much greater than those produced by a conical net with a wider aperture. The resulting lower sampling efficiency of the CALPS could explain, at least partly, the discrepancies in abundances recorded for all dominant taxonomic groups.

Active avoidance depends on the ability of the organisms to detect the presence of an incoming sampling device, in particular the "bow-wave" produced in front of the moving device (Clutter and Anraku, 1968), and on their swimming speed. The detection ability is species specific and a function of how an organism reacts to a range of visual, acoustic and hydrostatic stimuli (Fleminger and Clutter, 1965; Clutter and Anraku, 1968). In general, active avoidance of zooplankton increases with decreasing mouth opening size, mainly because the smaller the mouth opening, the lower the swimming speed is required for an animal to avoid capture (Clutter and Anraku, 1968). In this study, the effects of active avoidance are therefore expected to be greater for the CALPS than for the ring net. As larger organisms are generally faster, active avoidance could explain, at least partly, the bias towards larger individuals for some of the taxa captured by the ring net. The taxa that showed the largest difference in mean length between the ring net and CALPS samples also happened to be the largest (Fig. 6, Table II). Even if we omit the appendicularia, cnidaria and gammarid taxonomic groups, as these were captured on very few occasions, the taxa which were at least over 24% greater in the ring net include the decapod and polychaete larvae, *Calanus* spp. and *Centropages* spp. Apart from polychaete larvae, these taxa represented the largest individuals in samples from both devices which suggest that active avoidance behaviour explains part of the observed differences between the two sampling techniques.

Zooplankton sampling performance and the evaluation and intercomparison of sampling equipment have taken place since the introduction of quantitative techniques (Fraser, 1968; Wiebe and Benfield, 2003). Primary sources of errors across these techniques have been found to be escapement through the mesh, sampler avoidance and plankton patchiness. This was corroborated by the results of an in-depth intercomparison study on various net systems (Skjoldal *et al.*, 2013) which found that mesh size had a major influence on the abundance and zooplankton species composition; that towing speed could substantially increase extrusion of the smaller organisms through the net and that active avoidance is only important for the larger macrozooplankton. Passive avoidance due to sampler design was not considered. In this study, we have shown that surface samples collected by the CALPS and vertically integrated samples collected by the ring net provide similar results on the zooplankton community, but that different components of the community are sampled with different degrees of efficiency.

Our analysis suggests that avoidance of the CALPS as a result of its design (both passive and active avoidance) is likely to be the main factor explaining the higher abundances recorded by the ring net, but other factors

such as depth, area sampled, zooplankton patchiness, and behaviour of the animals are likely to play their part as well. Our results agree with other comparative studies between WP-2 ring nets and surface samplers such as the CPR (Clark *et al.*, 2001; John *et al.*, 2001; Richardson *et al.*, 2004), U-tow systems (Cook and Hays, 2001) and pump systems (Madurell *et al.*, 2012). The major difference between the CALPS and these systems is that the CALPS is an integral part of the ship and the sample is abstracted with a pump through a small opening rather than passed through an incoming net or larger opening. These specifications will certainly have an impact on the sampling efficiency of the system, but they still remain to be studied in depth to allow quantification. In a study comparing ichthyoplankton samples from the CUFES with those from ring nets, similar species compositions were found (Lelievre *et al.*, 2012). This is very relevant because of the similarities between the CUFES and the CALPS as both are integrated within the ship setup and their operation causes little disruption to the baseline survey program.

Another relevant comparison of sampling efficiency would be between the CALPS and the Continuous Plankton Recorder (CPR). This would allow for harmonization and standardization of the two methodologies and the integration of the datasets ultimately allowing the CALPS to fill the data gaps where the CPR is not deployed thereby increasing the resolution of dataset.

It is clear that the different properties of the CALPS and ring nets mean that they perform differently at measuring different specific parameters, and there are parameters for which they perform equality well, in line with results from other similar comparisons (Taggart and Leggett, 1984). Whilst the CALPS is not suitable for investigations that require accurate measures of abundance, eg accurate and vertically integrated zooplankton biomass for model calibration, it can identify and quantify changes in plankton communities as well as a ring net. In many circumstances, the spatial integration achieved by the CALPS might be more valuable in relation to a point sample, as a result of its integration of zooplankton patches. Previous comparative studies between CUFES and ring net samples have attempted to correct fish egg data from the CUFES, using non-linear modelling techniques, to estimate densities over the whole water column. For example, Lelievre *et al.* (2012) estimated total egg abundance in the water column from CUFES data with linear regression techniques including depth, bedstress and wind-induced mixing, which affected the vertical distribution of the eggs. Petitgas *et al.* (2006) converted fish egg concentrations from CUFES samples to vertically integrated abundances, using a one-dimensional vertical biophysical model, including egg properties, surface wind, tidal currents, temperature and salinity profiles, as model parameters. It might be possible to apply a similar approach to CALPS zooplankton samples in order to correct the data and make it more consistent with vertically integrated profiles as collected by ring nets. However, unlike fish eggs, zooplankton are not passive particles and their behaviours are species specific. Therefore, such a modelling task would be more complicated requiring additional species-specific parameters for calibration purposes.

CONCLUSION

The CALPS can identify and quantify changes in plankton communities and is therefore suited to describe broad geographic patterns in zooplankton community structure and diversity. Because of the challenge of reconciling economic efficiency with collection of robust scientific data, the adoption of integrated monitoring will constrain the types of sampling gear that can be used and therefore the properties monitored should be based on what the gear can achieve. A particular advantage of the CALPS over more traditional vertical sampling methods is that it can be integrated within existing multidisciplinary surveys at little extra cost and without requiring additional survey time. In order to optimize zooplankton monitoring, cost associated with post-cruise processing and analysis of the large number of samples produced could be dealt with the development of image analysis systems that can be used on-board the survey vessel such as the LiZA/PIA system (Culverhouse, 2015). Because no single device is able to sample all the zooplankton components at any one time, multidisciplinary programs studying marine ecosystem structure and dynamics often use nets designed to sample particular size fractions in combination with video and/or acoustic techniques (Postel *et al.*, 2007; Lara-Lopez and Neira, 2008; Lavery *et al.*, 2010) thus allowing a large spatial coverage and a relative high resolution in horizontal and vertical planes. Such a set-up would complement the data obtained from CALPS samples. All the above features make the CALPS a particularly useful tool as part of integrated monitoring of environmental status to underpin policy areas such as the MSFD.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We acknowledge the considerable efforts of the officers and crew of RV Cefas Endeavour involved in the PELTIC survey in October 2014. We also acknowledge the efforts of Thomas Maes in obtaining the original funding for the construction of the CALPS and working along Jena Engineering at the design stage.

FUNDING

UK Government Department for Environment, Food and Rural Affairs DEFRA under grant ME5319 (Hierarchical Assessment of Zooplankton According to Risks and for MSFD Descriptors: HAZARD), with additional support from project POSEIDON, DEFRA grant MF1112 (Pelagic Ocean Science: Ecology and Interconnectivity of Diverse Ocean Networks).

REFERENCES

Albaina, A., Irigoien, X., Aldalur, U., Boyra, G., Santos, M. and Estonba, A. (2014) Macrozooplankton predation impact on anchovy (*Engraulis encrasicolus*) eggs mortality at the Bay of Biscay shelf break spawning centre. *ICES J. Mar. Sci.*, 72, 1370–1379.

Batten, S. D., Edwards, M. and Beaugrand, G. (2013) All plankton sampling systems underestimate abundance: Response to "Continuous plankton recorder underestimates zooplankton abundance" by J.W. Dippner and M. Krause. *J. Mar. Syst.*, 128, 240–242.

Bograd, S. J., Checkley, D. A. and Wooster, W. S. (2003) CalCOFI: a half century of physical, chemical, and biological research in the California Current System. *Deep Sea Res. II*, 50, 2349–2353.

Borja, A., Elliott, M., Andersen, J. H., Cardoso, A. C., Carstensen, J., Ferreira, J. G., Heiskanen, A.-S., Marques, J. C. *et al.* (2013) Good Environmental Status of marine ecosystems: what is it and how do we know when we have attained it? *Mar. Pollut. Bull.*, 76, 16–27.

Brodeur, R. D. and Ware, D. M. (1992) Long-term variability in zooplankton biomass in the subarctic Pacific Ocean. *Fish. Oceanogr.*, 1, 32–39.

Checkley, D. Jr., Ortner, P. B., Settle, L. R. and Cummings, S. R. (1997) A continuous, underway fish egg sampler. *Fish. Oceanogr.*, 6, 58–73.

Clark, R. A., Frid, C. L. J. and Batten, S. (2001) A critical comparison of two long-term zooplankton time series from the central-west North Sea. *J. Plankton Res.*, 23, 27–39.

Clarke, K. R. and Warwick, R. M. (1994) Similarity-based testing for community pattern: the two-way layout with no replication. *Mar. Biol.*, 118, 167–176.

Clutter, R. I. and Anraku, M. (1968) Avoidance of samplers. In Tranter, D. J. (ed), *Monographs on Oceanographic Methodology, Vol. 2: Zooplankton Sampling*. UNESCO, Paris, pp. 57–76.

Cook, K. B. and Hays, G. C. (2001) Comparison of the epipelagic zooplankton samples from a U-Tow and the traditional WP2 net. *J. Plankton Res.*, 23, 953–962.

Culverhouse, P. F. (2015) An instrument for rapid mesozooplankton monitoring at ocean basin scale. *J. Mar. Biol. Aquacult.*, 1, 1–11.

Dixon, P. and Robertson, A. I. (1986) A compact, self-contained zooplankton pump for use in shallow coastal habitats: design and performance compared to net samples. *Mar. Ecol. Prog. Ser.*, 32, 97–100.

Edwards, M. and Richardson, A. J. (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, 430, 881–884.

Fleminger, A. and Clutter, R. I. (1965) Avoidance of towed nets by zooplankton. *Limnol. Oceanogr.*, 10, 96–104.

Fraser, J. H. (1968) The history of plankton sampling. In Tranter, D. J. (ed), *Monographs on Oceanographic Methodology, Vol. 2: Zooplankton Sampling*. Vol. 2. UNESCO, pp. 11–18.

Gorsky, G., Ohman, M. D., Picheral, M., Gasparini, S., Stemmann, L., Romagnan, J.-B., Cawood, A., Pesant, S. *et al*. (2010) Digital zooplankton image analysis using the ZooScan integrated system. *J. Plankton Res.*, 32, 285–303.

Grosjean, P., Picheral, M., Warembourg, C. and Gorsky, G. (2004) Enumeration, measurement, and identification of net zooplankton samples using the ZOOSCAN digital imaging system. *ICES J. Mar. Sci.*, 61, 518–525.

Harrop, V. and Edwards, M. (2014) Multidecadal Atlantic climate variability and its impact on marine pelagic communities. *J. Mar. Syst.*, 133, 55–69.

Hobday, A. J., Okey, T. A., Poloczanska, E. S., Kunz, T. J., Richardson, A. J. (eds) (2006) Impacts of climate change on Australian marine life. Part C. Literature Review. *Report to the Australian Greenhouse Office*, Canberra, Australia, 165 pp.

ICES (2015) *Manual for International Pelagic Surveys (IPS)*. Series of ICES Survey Protocols SISP 9 – IPS. 92 pp.

John, E. H., Batten, S. D., Harris, R. P. and Hays, G. C. (2001) Comparison between zooplankton data collected by the Continuous Plankton Recorder survey in the English Channel and by WP-2 nets at station L4, Plymouth (UK). *J. Sea Res.*, 46, 223–232.

Lampert, W. (1989) The adaptive significance of diel vertical migration of zooplankton. *Funct. Ecol.*, 3, 21–27.

Lara-Lopez, A. and Neira, F. J. (2008) Synchronicity between zooplankton biomass and larval fish concentrations along a highly flushed Tasmanian estuary: assessment using net and acoustic methods. *J. Plankton Res.*, 30, 1061–1073.

Lauria, V., Attrill, M. J., Brown, A., Edwards, M. and Votier, S. C. (2013) Regional variation in the impact of climate change: evidence that bottom-up regulation from plankton to seabirds is weak in parts of the Northeast Atlantic. *Mar. Ecol. Prog. Ser.*, 488, 11–22.

Lavery, A. C., Chu, D. and Moum, J. N. (2010) Measurements of acoustic scattering from zooplankton and oceanic microstructure using a broadband echosounder. *ICES J. Mar. Sci.*, 67, 379–394.

Lelievre, S., Vaz, S. and Fox, C. (2012) The effectiveness of the continuous underway fish egg sampler compared with standard vertical plankton hauls for sampling the eggs of demersal species in the southern North Sea. *Fish. Oceanogr.*, 21, 109–124.

Mackas, D. L., Denman, K. L. and Abbott, M. R. (1985) Plankton patchiness: biology in the physical vernacular. *Bull. Mar. Sci.*, 37, 652–674.

Madurell, T., Olariaga, A. and Gili, J.-M. (2012) Submarine coupled multi-filtration pump. *J. Plankton Res.*, 34, 1078–1085.

Møhlenberg, F. (1987) A submersible net-pump for quantitative zooplankton sampling; comparison with conventional net sampling. *Ophelia*, 27, 101–110.

Petitgas, P., Magri, S. and Lazure P. (2006) One-dimensional biophysical modelling of fish egg vertical distributions in shelf seas. *Fish. Oceanogr.*, 15, 413–428.

Pitois, S. G., Lynam, C. P., Jansen, T., Halliday, N. and Edwards, M. (2012) Bottom-up effects of climate on fish populations: data from the Continuous Plankton Recorder. *Mar. Ecol. Prog. Ser.*, 456, 169–186.

Postel, L., Da Silva, A. J., Mohrholz, V. and Lass, H.-U. (2007) Zooplankton biomass variability off Angola and Namibia investigated by a lowered ADCP and net sampling. *J. Mar. Syst.*, 68, 143–166.

Richardson, A. J. (2008) In hot water: zooplankton and climate change. *ICES J. Mar. Sci.*, 65, 279–295.

Richardson, A. J., John, E. H., Irigoien, X., Harris, R. P. and Hays, G. C. (2004) How well does the continuous plankton recorder (CPR) sample zooplankton? A comparison with the Longhurst Hardy Plankton Recorder (LHPR) in the northeast Atlantic. *Deep Sea Res. I*, 51, 1283–1294.

Scherer, C., Gowen, R. J., Tett, P., Mcquatters-Gollop, A., Forster, R., Bresnan, E., Cook, K., Atkinson, A. *et al.* (2014) A final report of the Lifeform and State Space project Prepared for The Department of Environment. *Food Rural Affairs*, 71.

Shephard, S., Van Hal, R., De Boois, I., Birchenough, S. N. R., Foden, J., O'connor, J., Geelhoed, S. C. V. *et al*. (2015) Making progress towards integration of existing sampling activities to establish Joint Monitoring Programmes in support of the MSFD. *Mar. Policy*, 59, 105–111.

Skjoldal, H. R., Wiebe, P. H., Postel, L., Knutsen, T., Kaartvedt, S. and Sameoto, D. D. (2013) Intercomparison of zooplankton (net) sampling systems: results from the ICES/GLOBEC sea-going workshop. *Prog. Oceanogr.*, 108, 1–42.

Smyth, T., Atkinson, A., Widdicombe, S., Frost, M., Allen, I., Fishwick, J., Queiros, A., Sims, D. W. *et al*. (2015) The Western channel observatory. *Prog. Oceanogr.*, 137, 335–341.

Sono, S., Moloney, C. and Van Der Lingen, C. (2009) Assessing the utility of a continuous, underway fish egg sampler (CUFES) for sampling zooplankton. *Afr. J. Mar. Sci.*, 31, 181–189.

Taggart, C. T. and Leggett, W. C. (1984) Efficiency of large-volume plankton pumps, and evaluation of a design suitable for deployment from small boats. *Can. J. Fish. Aquat. Sci.* 41, 1428–1435.

Tranter, D. J. and Smith, P. E. (1968) Filtration performance. In: Tranter, D. J. (ed) *Zooplankton Sampling. Part I. Reviews on Zooplankton Sampling Methods*. Vol. 2. UNESCO, pp. 27–56.

Wiebe, P. H. and Benfield, M. C. (2003) From the Hensen net toward four-dimensional biological oceanography. *Prog. Oceanogr.*, 56, 7–136.

Weber, E. D., Chao, Y., Chai, F. and Mcclatchie, S. (2015) Transport patterns of Pacific sardine Sardinops sagax eggs and larvae in the California Current System. *Deep Sea Res. Part I Oceanogr. Res. Pap.*, 100, 127–139.

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bull.*, 1, 80–83.