

# Distributions of physonect siphonulae in the Gulf of Maine and their potential as important sources of acoustic scattering

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**Abstract:** The distributions of siphonulae stages of physonect siphonophores were mapped in Wilkinson, Jordan, and Georges basins of the Gulf of Maine using a video plankton recorder. Siphonulae are often overlooked in net samples and our optical survey appears to be the first in situ investigation of these organisms. Siphonulae were distributed at mid-depths in narrow horizontal layers, suggesting potential control of their buoyancy. The siphonulae possessed gas-filled pneumatophores with diameters of 0.1–0.4 mm. Pneumatophore diameters appeared to be similar over their entire sampled depth range, suggesting that siphonulae may be capable of regulating the pressure of gas within the pneumatophore to maintain a constant volume. The dimensions of the siphonulae pneumatophores placed them near the acoustic resonance region for scattering at 43 kHz when near the surface and 120 kHz when at depth. Theoretical estimates of the acoustical target strengths of gas bubbles of sizes corresponding to the sizes of our measured pneumatophores produced relatively strong backscatter. Layers of siphonulae corresponded to regions of high acoustical backscatter at 120, 200, and 420 kHz.

**Résumé :** Un système d'enregistrement vidéo du plancton nous a permis de décrire les répartitions des stades siphonulas des siphonophores physonectes dans les bassins Wilkinson, Jordan et Georges du golfe du Maine. Les larves siphonulas sont souvent négligées dans les échantillons au filet et notre inventaire optique semble être la première étude in situ de ces organismes. Les siphonulas se distribuent aux profondeurs moyennes en minces couches horizontales, ce qui fait croire qu'elles ont le pouvoir de contrôler leur flottabilité. Les siphonulas possèdent des pneumatophores de diamètres de 0,1 à 0,4 mm et remplis de gaz. Les diamètres des pneumatophores semblent être semblables dans toute la gamme des profondeurs échantillonnées, ce qui paraît montrer que les siphonulas sont capables de régler la pression gazeuse dans leurs pneumatophores de façon à maintenir le volume constant. Les tailles des pneumatophores des siphonulas avoisinent la région de résonance acoustique de diffusion à 43 kHz près de la surface et à 120 kHz en profondeur. Les estimations théoriques des forces acoustiques de cibles de bulles gazeuses de tailles correspondant aux tailles mesurées chez les pneumatophores produisent une rétrodiffusion relativement forte. Les couches de siphonulas correspondent à des régions d'importante rétrodiffusion acoustique à 120, 200 et 420 kHz.

[Traduit par la Rédaction]

## Introduction

Estimation and prediction of the distributions and abundances of marine organisms are central goals of biological oceanography and constitute specific objectives of programs such as U.S. GLOBEC (Global Ocean Ecosystem Dynamics; U.S. GLOBEC 1992). In the case of marine zooplankton, knowledge of where and when zooplankton taxa occur re-

mains an essential precursor for understanding the population dynamics of both zooplankton and higher trophic levels, particularly fish. The pursuit of these goals has been particularly challenging for oceanographers studying zooplankton because of horizontal and vertical patchiness, avoidance, gear-selectivity, and the fragility of many taxa. Although much has been learned about subadult and adult stages of specific zooplankton, it is a fair generalization that our cur-

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rent understanding of the ecology of larval and juvenile stages is far more limited.

Physonect siphonophores are a suborder of broadly distributed, colonial, gelatinous organisms of considerable ecological importance because of their capacity for predation on ichthyoplankton and invertebrate zooplankton (Barham 1963). Physonect siphonophores are generally distinguished by the presence of both a gas-filled inclusion called a pneumatophore and a series of one or more medusoid swimming bells called nectophores that are used for propulsion (Totton 1965; Mackie et al. 1987). These colonial organisms are also of potential interest to those undertaking acoustic surveys of the water column because of their disproportionately strong acoustic returns relative to their biomass (Barham 1963; Stanton et al. 1994; Warren et al. 2001). The gas-filled pneumatophore is primarily responsible for the strong acoustic echo from siphonophores, particularly at acoustical frequencies near the resonance frequency of the gas bubble (Stanton et al. 1994, 1998a, 1998b). Although physonect colonies are known to be strong acoustic scatterers, their contribution to the scattering is easily overlooked during "sea-truthing" surveys because large colonies may be agile swimmers (Barham 1963; Rogers et al. 1978) capable of avoiding many nets or they are severely damaged during the collection process (Totton 1965; Hamner et al. 1975; Warren et al. 2001). When colonies are collected by larger nets such as the multiple opening/closing net and environmental sensing system (MOCNESS), they are often shredded (Greene et al. 1998) and enumeration is based on the presence of recognizable remnants such as pneumatophores and nectophores. Although some net systems such as the Reeve net (Reeve 1981) have been designed to minimize such damage (see Wiebe and Benfield (2001, 2003) for a summary of these and other net systems), our understanding of the ecology of gelatinous plankton has been limited to a large extent by sampling difficulties. The development of in situ observational techniques (e.g., occupied and unoccupied submersibles) and remote image-forming optical survey systems such as the video plankton recorder (VPR; Davis et al. 1992) has substantially advanced our understanding of the ecology of gelatinous plankton.

Physonect siphonophores reproduce by producing sperm and eggs within gonophores. Fertilized eggs are released into the water column where they pass through planulus larval stages and develop into the siphonula stage (Mackie et al. 1987). Although there is some question as to whether siphonulae are true larvae (Sherlock and Robison 2000), we follow their convention and refer to them as a larval stage. Early in the development of the siphonula stage, physonect genera such as *Nanomia* begin to secrete gas into the pneumatophore (Mackie et al. 1987; Sherlock and Robison 2000). With the development of the pneumatophore, the small siphonula stages may be detected acoustically, especially when ensonified at frequencies near the resonance frequency of the gas inclusion. It is well established that adult physonect siphonophores produce strong acoustic echoes near the resonance frequency of the pneumatophore (Barham 1963; Stanton et al. 1994; Warren et al. 2001). Nothing has been reported about the potential for smaller siphonophore stages to scatter sound. Gas-bearing siphonulae are far less likely than adult colonies to be enumerated during net sam-

pling because of their small size and amorphous appearance in preserved samples. If siphonulae produce strong acoustical returns and are difficult to quantify with traditional samplers, it may be difficult to explain the source of strong acoustical scattering in regions where they are abundant.

During analysis of VPR images collected during surveys in the Gulf of Maine, we observed small, unidentified, tentaculate organisms that were subsequently identified as siphonula larvae of physonect siphonophores (Rob Sherlock and Bruce Robison, Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, personal communication). Although the pneumatophores of these siphonulae appeared to be smaller than those of adult colonies by approximately an order of magnitude, our estimates of their acoustical target strengths suggested that these small pneumatophores were still producing relatively strong echoes, especially at the lower portion of our frequency range (43 and 120 kHz) because their gas inclusions were at, or near, resonance. The objectives of this study were (1) to quantify the spatial distributions of physonect siphonulae, (2) to determine the ranges of environmental conditions associated with their distributions, and (3) to estimate their contribution to measured acoustical scattering.

## Materials and methods

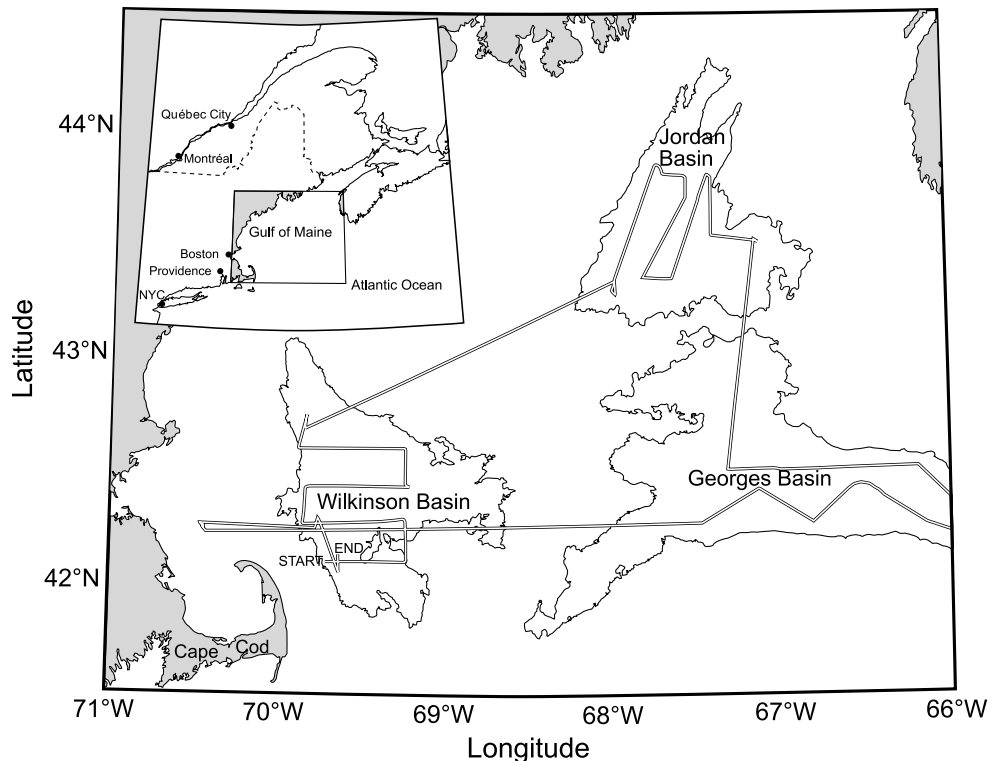
### Study area

Our data were collected during a research cruise aboard the R/V *Endeavor* in the Gulf of Maine from 4 to 13 December 1999. This cruise was part of the U.S. GLOBEC program and our primary objective was to quantify the distribution of the diapausing copepod *Calanus finmarchicus* and its primary invertebrate predators — physonect siphonophores such as *Nanomia cara* and the euphausiid *Meganycitophanes norvegica* — in Wilkinson, Jordan, and Georges basins of the Gulf of Maine (Fig. 1).

### Oceanographic surveying

Our surveys were conducted using a high-speed, deep-towed system called BIOMAPER II (bio-optical, multi-frequency acoustical, and physical environmental recorder, a custom-built system constructed by the Woods Hole Oceanographic Institution, Woods Hole, Mass.) that was equipped with five echosounder pairs operating at 43, 120, 200, 420, and 1000 kHz, a single-camera VPR, and a suite of environmental and bio-optical sensors (Wiebe et al. 1997, 2002; Austin et al. 1998). Each pair of transducers on BIOMAPER II was oriented so that one aimed upwards and the other downwards. During surveys, BIOMAPER II was lowered and hauled back in a saw-tooth trajectory, called a towyo, between the surface and within 10–20 m of the bottom to provide maximal coverage of the water column for most sensors, including the highest acoustical frequencies. Vertical descent and ascent rates were typically 5 m·min<sup>-1</sup> at a horizontal velocity of 6–8 knots. The transducer pairs fired in a cyclical sequence with a ping interval of 2.5 s. Echo integration was performed at the end of each 12-s cycle to provide estimates of volume backscattering, for each frequency, in 1 m thick depth bins to the maximum effective range for each frequency (43 kHz, 200 m; 120 kHz, 200 m; 200 kHz, 150 m; 420 kHz, 100 m; 1000 kHz, 35 m). In this study, we

**Fig. 1.** The track line of the R/V *Endeavor* in the Gulf of Maine during survey operations conducted from 4 to 13 December 1999. The primary deep basins of the Gulf of Maine (Wilkinson Basin, Jordan Basin, and Georges Basin) are delimited with the 200-m isobaths. The inset map in the upper left corner illustrates the location of the study area within the New England – Maritimes region.



include 43, 120, 200, and 420 kHz data. The limited range of the 1000 kHz transducers made it difficult to interpret echogram patterns and results from 1000 kHz were omitted.

The VPR is an underwater high-magnification video system (Davis et al. 1992) that collects images of plankton within the water column using a high-speed strobe and video camera. Our VPR was a single-camera model that was mounted on the upper front end of BIOMAPER II. It imaged a volume of 5.1 mL at a rate of 60 Hz ( $18.43 \text{ L} \cdot \text{min}^{-1}$ ). Video data were transmitted to the surface via an electro-optical tow cable, stamped with a time code derived from global positioning system data, and stored on S-VHS format videotape.

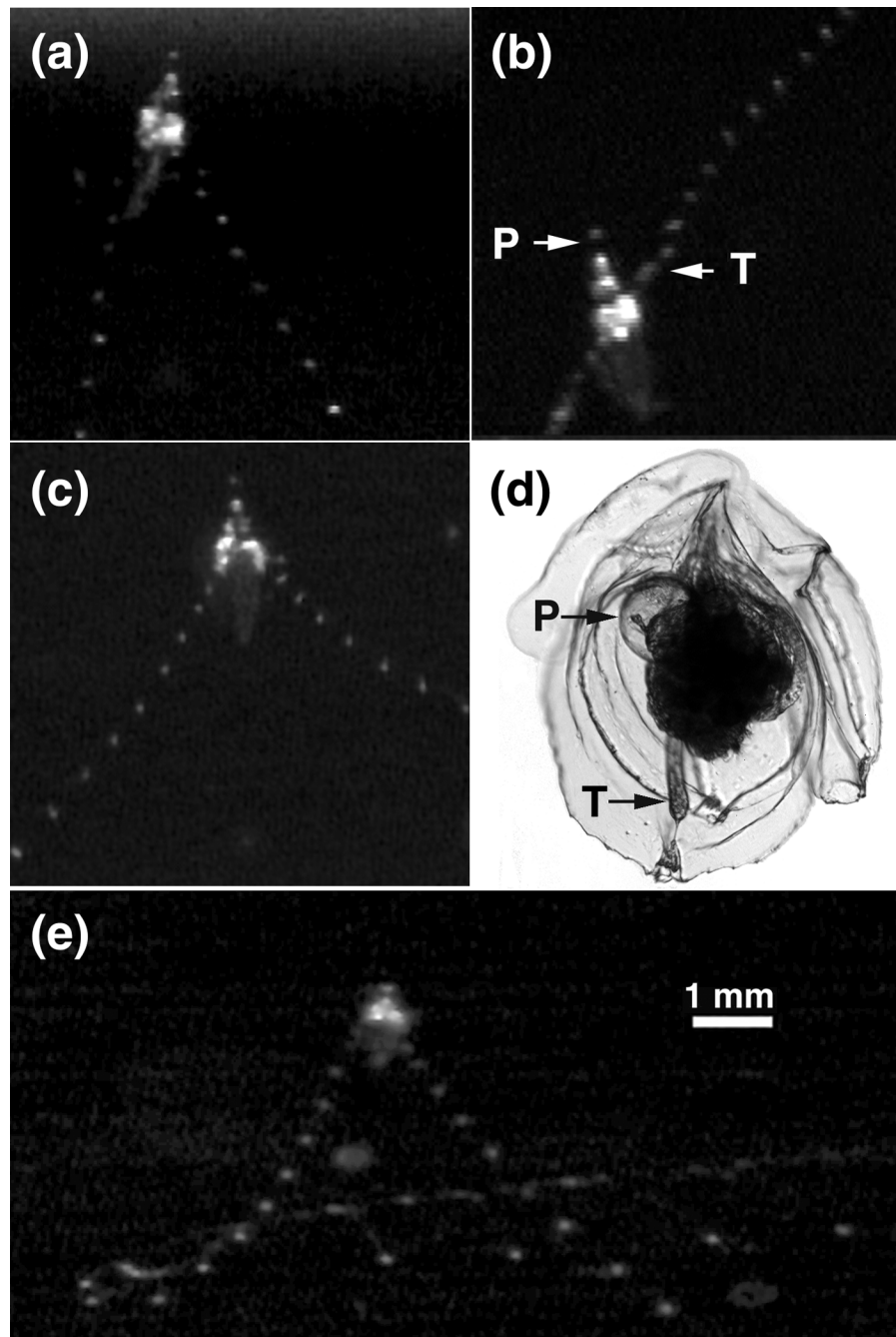
### Data analysis

Videotapes were postprocessed after the cruise using an image processor (Imaging Technology Inc., Bedford, Mass.) that digitized and then examined each video field for targets that met user-defined criteria for size, brightness, and focus. Targets that met these criteria were termed regions of interest (ROIs) and were written to disk using a filename that represented the elapsed time in milliseconds since midnight (GMT) that each ROI was detected. Because prior testing indicated that one set of ROI extraction parameters was not optimal for all zooplankton taxa, each tape was processed three times with different extraction parameters. Graphical image browsing software (ThumbsPlus 4.10; Cerious Software Inc., Charlotte, N.C.) was then used to eliminate duplicate ROIs and to sort ROIs into taxonomic categories. ROIs containing siphonulae (Fig. 2) were separated from all other

targets. Then the time of detection of each siphonula was used to determine the depth, latitude, longitude, and environmental conditions of each individual based on the BIOMAPER II trajectory and environmental sensing system data. The diameters of pneumatophores in ROIs that contained visible pneumatophores (154 individuals out of a total of 175 siphonulae detected by the image processor) were measured using a Matlab program (The Mathworks Inc., Natick, Mass.). The densities of siphonulae were estimated within 10 m thick depth bins along the BIOMAPER II track line by summing the total number of siphonulae detected while BIOMAPER II passed through each 10-m depth stratum and then dividing by the volume of water surveyed in the same interval by the VPR. The volume of water imaged by the VPR within each 10-m stratum varied as a function of the vessel velocity, BIOMAPER II descent rate, and prevailing currents. Based on a 4-h section of tape in Jordan Basin that contained three typical towys, the mean volume imaged within each 10 m thick depth stratum was 62.0 L. Each density estimate based on the imaged volume was then scaled up to a density within a standard volume ( $n \cdot \text{m}^{-3}$ ).

A 4-h section of tape from the end of our survey of Jordan Basin collected from 1118 to 1522 on 7 December 1999 (341.4713–341.6405 in Julian day format) was also examined manually to determine the extraction efficiency of the image processor for siphonulae ROIs. Each of the two S-VHS tapes were viewed at slow speed using an editing deck, and the S-video output from the deck was routed into a laptop computer (Macintosh Powerbook G3; Apple Computer Inc., Cupertino, Calif.) using a video capture card (Irez Inc.,

**Fig. 2.** (a–c) Examples of larval physonect siphonophores from video plankton recorder (VPR) images collected by BIOMAPER II in the Gulf of Maine during December 1999. (d) A preserved siphonula collected with a 1-m<sup>2</sup> MOCNESS net from Jordan Basin at night during the same cruise as the VPR images. The pneumatophore (P) and tentacle (T) are indicated. (e) A VPR image that shows a single siphonula with tentacles extended in a fishing posture containing scaling information.



Scottsdale, Ariz.) and video digitizing software (Irez ReelEyes; Irez Inc.). A still image of each manually detected siphonula was stored as a TIFF- or JPEG-formatted image using the time code as a file name. The file names of all manually located siphonulae were read into Matlab and converted to local time (Julian day with January 1 = day 1). The positions of these siphonulae were then superimposed over the acoustics record from BIOMAPER II to evaluate whether the abundances of siphonulae were associated with patterns of elevated acoustical scattering. Finally, the num-

ber of siphonulae detected manually was compared with the number detected semiautomatically to determine the extraction efficiency of the image processor.

The vertical distributions of siphonulae were estimated in 10-m depth bins beginning at the surface and extending to the maximum depth surveyed. Vertical distributions were estimated during the day and night for each basin in the Gulf of Maine. Wilkinson Basin was visited twice during the cruise, and both surveys in that area were examined separately because several days separated the two surveys. Day

distributions included all siphonulae detected from 1 h after the average time of sunrise (0650 local time) until 1 h before the average time of sunset (1600 local time). Night distributions included all siphonulae detected from 1 h after sunset until 1 h before sunrise.

Periodic net samples were collected with a 1-m<sup>2</sup> MOCNESS sampler equipped with 335 µm mesh nets. Samples from one net cast collected in Jordan Basin between 2255 and 2308 on 6 December 1999 (Julian day 340.9549–340.9639) were examined for siphonulae. Although the MOCNESS is not likely to provide a quantitative record of siphonulae distributions, it does retain some fraction of the siphonulae that enter the nets. We measured the diameters of all preserved siphonula pneumatophores that were recovered from each of the eight nets with an optical micrometer and a microscope to compare the size distributions of net-sampled siphonulae with those measured from VPR images.

The exact modal series solution to the wave equation for scattering from a fluid sphere (Anderson 1950) was used to predict the scattering from each measured siphonula pneumatophore (from VPR images). Details of this methodology and associated assumptions are provided in Appendix A.

We estimated the contribution to volume scattering at 43, 120, 200, and 420 kHz by siphonulae for the 4-h section of the acoustic record from Jordan Basin that corresponded to the visually examined section of videotape. The acoustic data were separated into three towyo sections. Within each towyo section, the mean volume backscattering coefficient ( $\bar{s}_v$ ) was estimated by averaging all measured  $s_v$  values within each 10 m thick stratum from 0 to 200 m. The forward problem (prediction of acoustic scattering from VPR-derived numbers and sizes of individuals) was then solved in the following manner. For each 10-m stratum, the predicted backscattering cross section of every pneumatophore was estimated for each of the four acoustical frequencies by accounting for the density of siphonulae in the corresponding size class per cubic metre within the stratum (Appendix A). This yielded an estimate of the contribution to the measured volume backscattering coefficient ( $s_v$ ) by siphonulae in each depth stratum. The percent contribution of siphonulae to measured volume backscattering coefficients in each depth stratum within each of the three towyos was then estimated

$$\text{as } \frac{s_{v,\text{siphonulae}}}{\bar{s}_v} \times 100.$$

## Results

### Spatial distributions

Of 175 siphonulae detected by the image processing system from survey operations in the Gulf of Maine, all but three were found in the waters over one of the three surveyed basins, and the remainder were imaged during transit from one basin to the next. The distributions of siphonulae were highly patchy. Some dense patches were encountered in surface waters of Wilkinson Basin; however, most siphonulae were distributed at mid-depths in all three basins (Fig. 3) and often formed well-defined horizontal layers. Siphonulae tended to be distributed deepest in Georges Basin and shallowest in Wilkinson Basin (Figs. 3, 4).

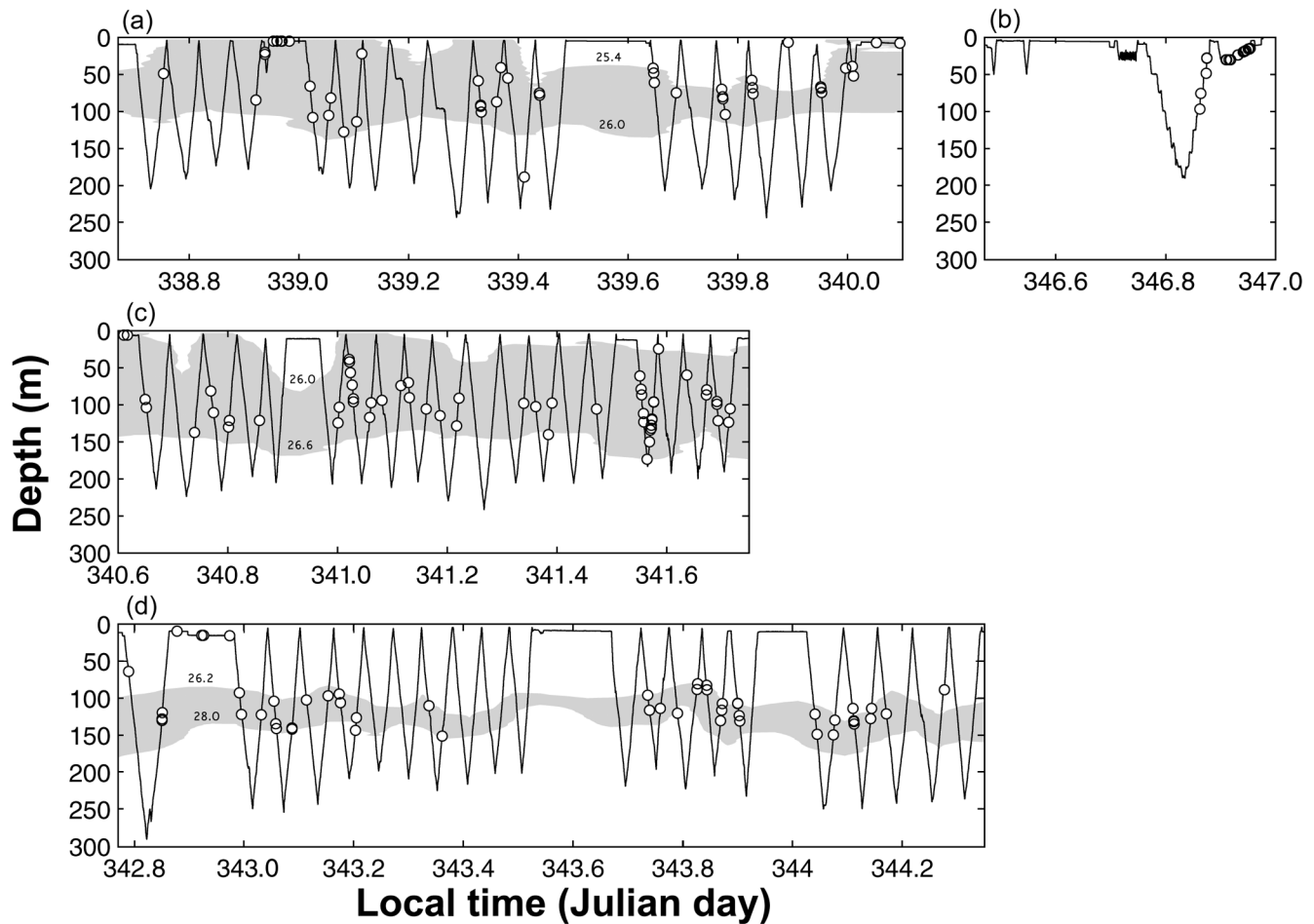
Siphonulae were small and rather faint images on the videotape. Consequently, our image processor had a relatively low extraction efficiency of 36.7% based on comparisons between ROIs detected by the image processor and our manual examination of 4 h of tape. Thus, the 175 animals detected by the processor likely represented the presence of over 450 individuals. Estimates of the densities of siphonulae in the water column based on the manually detected data set indicated that siphonulae could be present in relatively high numbers (up to 96.6 siphonulae·m<sup>-3</sup>), although the mean density of 8.2 siphonulae·m<sup>-3</sup> was considerably lower.

The existence of a diel vertical migration pattern was not clearly supported in all three basins (Fig. 4). Wilkinson Basin was the only region in which differences in the day and night distributions suggested a pattern of vertical migration. During the day, siphonulae were detected in the depth range of 40.7 to 188.5 m (median of 75.4 m, mean of 78.5 m). At night during the primary survey, siphonulae were observed in the depth range of 5.1 to 127.4 m (median of 65.9 m, mean of 54.8 m) in a strongly bimodal distribution with one mode in the depth range 70 to 80 m and a second in the upper 10 m (Fig. 4). During subsequent operations in Wilkinson Basin that were conducted after dark, the vertical distribution was strongly skewed towards the surface with most siphonulae in the upper 10–20 m of the water column (Fig. 4). In Jordan Basin, siphonulae displayed similar day and night distributions (Fig. 4), although there was some indication that animals were present at shallower depths and that the center of the distribution rose during the night. During the day, they were detected in the depth range of 24.4 to 173.3 m in a unimodal distribution (median of 104.7 m, mean of 106.4 m; Fig. 4). At night, siphonulae were detected from 5.1 to 149.5 m in a unimodal distribution (median of 98.1 m, mean of 98.0 m). Only two siphonulae were detected during the day over Georges Basin, and both were present below 100 m (Fig. 4). At night, siphonulae were present in the depth range of 9.5 to 149.5 m. Although a few animals were present at the surface, most were concentrated at depth (median of 116.1 m, mean of 107.7 m).

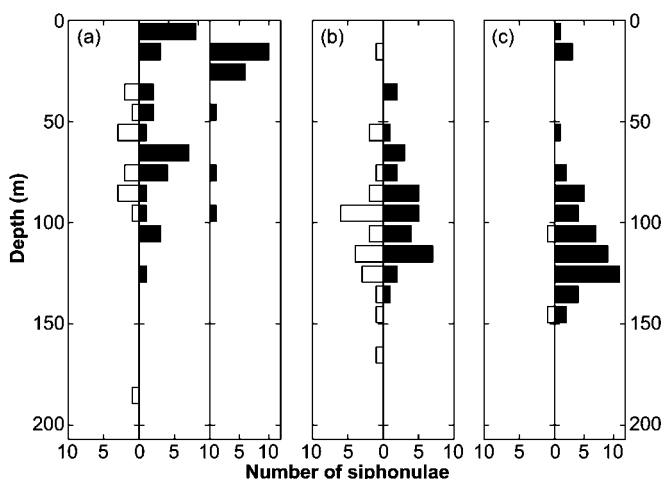
### Physical conditions associated with siphonulae

Siphonulae were most abundant in waters warmer than 8.25°C in all three basins (Fig. 5). The mean temperatures of waters where siphonulae were detected were 8.85, 9.82, and 9.89°C for Wilkinson, Jordan, and Georges basins, respectively. Salinity appeared to have less influence on the distributions of siphonulae than did temperature. Wilkinson Basin was fresher than the other two basins and siphonulae in Wilkinson were most abundant in the range of 32.5 to 33.5‰ (mean of 33.03‰; Fig. 5). In the more saline Jordan and Georges basins, most siphonulae were found in the range of 33.75 to 34.75‰ waters (Jordan mean of 34.09‰, Georges mean of 34.24‰; Fig. 5). In Georges Basin, most siphonulae were located within the 26.2–28.0 σ-theta density contours, whereas in Wilkinson and Jordan basins, most siphonulae were located within a narrower range of less dense water that occupied a broader extent of the water column (Fig. 3; Wilkinson range of 25.4 to 26.0 σ-theta and Jordan range of 26.0 to 26.6 σ-theta). There was no consistent relationship between fluorescence and the distributions of siphonulae in any of the basins. In general, siphonulae were more common

**Fig. 3.** Distributions of physonect siphonulae detected with the video plankton recorder in Wilkinson Basin during (a) the primary survey and (b) the secondary survey, (c) Jordan Basin, and (d) Georges Basin. The shaded regions delimit the range of density isopycnals ( $\sigma$ -theta) that contained the majority of siphonulae in each basin. There was insufficient coverage of the water column during the secondary survey of Wilkinson basin to contour the isopycnals.



**Fig. 4.** Vertical distributions of siphonulae during day (sunrise + 1 h to sunset - 1 h; open bars) and night (sunset + 1 h to sunrise - 1 h; solid bars) in (a) Wilkinson Basin, (b) Jordan Basin, and (c) Georges Basin. There are two nighttime distributions for Wilkinson Basin because it was surveyed at the beginning of the cruise and limited survey operations were conducted again during nighttime at the end of the cruise.

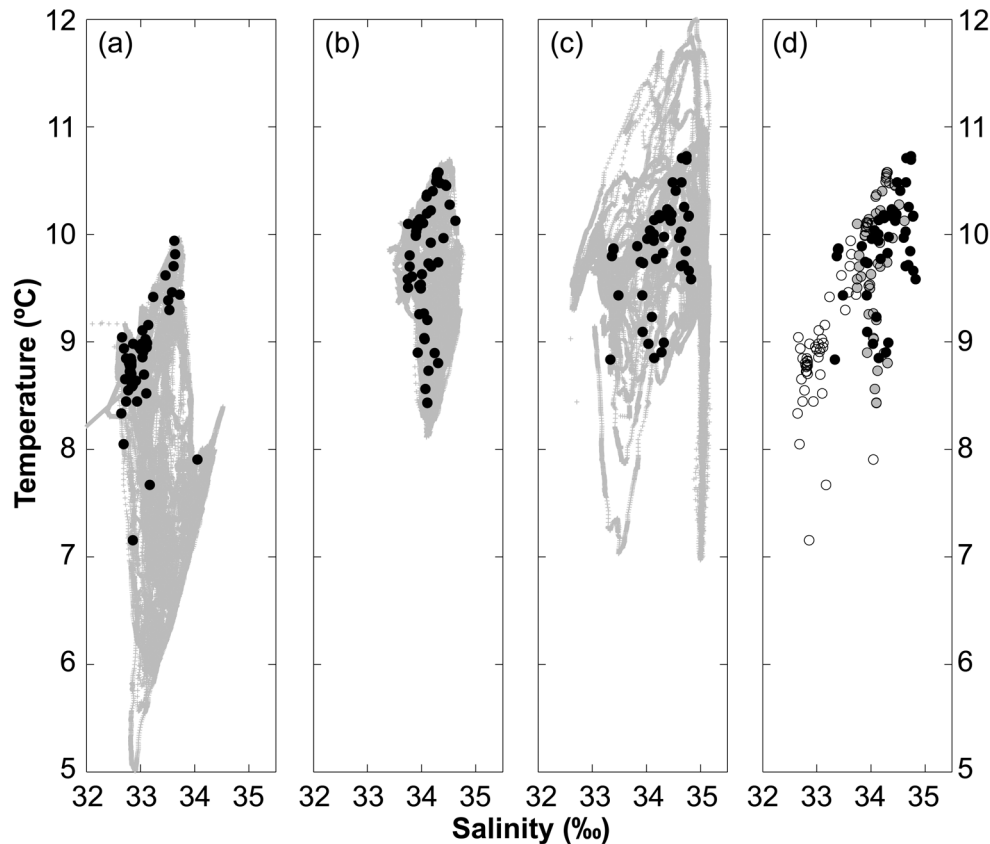


at the lower end of the range of fluorescence values that occurred in the surveyed zone of each basin.

#### Comparisons of predicted and measured acoustic scattering by siphonulae

The size distribution of 154 measured siphonulae pneumatophores was approximately normally distributed with a mean diameter of 0.21 mm and a median of 0.20 mm (Fig. 6a). A regression of pneumatophore diameter on depth was nonsignificant ( $R^2 = 0.003$ ,  $p = 0.46$ ), and the pneumatophore diameters did not appear to change substantially with depth (Fig. 6b). The mean diameter of pneumatophores from 229 siphonulae collected from Jordan Basin with a 1-m<sup>2</sup> MOCNESS during the late evening (2255–2308 local time) of day 340 was 0.21 mm (range of 0.1 to 0.4 mm). The pneumatophores from the MOCNESS nets had a size distribution that was similar to that of the VPR with some elevated frequencies of smaller diameters that was consistent with postcollection loss of gas. The predicted acoustical target strength (TS) of siphonulae pneumatophores was sensitive to changes in the diameter of the gas bubble (Fig. 6c) and hydrostatic pressure (Fig. 6d). As the pneumatophore diameter decreased, the resonance frequency increased. At 100-m depth, larger pneumatophores with

**Fig. 5.** Temperature–salinity ( $T$ – $S$ ) data (shaded areas) from BIOMAPER II surveys in (a) Wilkinson, (b) Jordan, and (c) Georges basins overlaid with the  $T$ – $S$  conditions measured at points where siphonulae were detected (●). (d) The combined  $T$ – $S$  conditions from all observations of siphonulae in the three basins: Wilkinson (○), Jordan (shaded circles), and Georges (●).



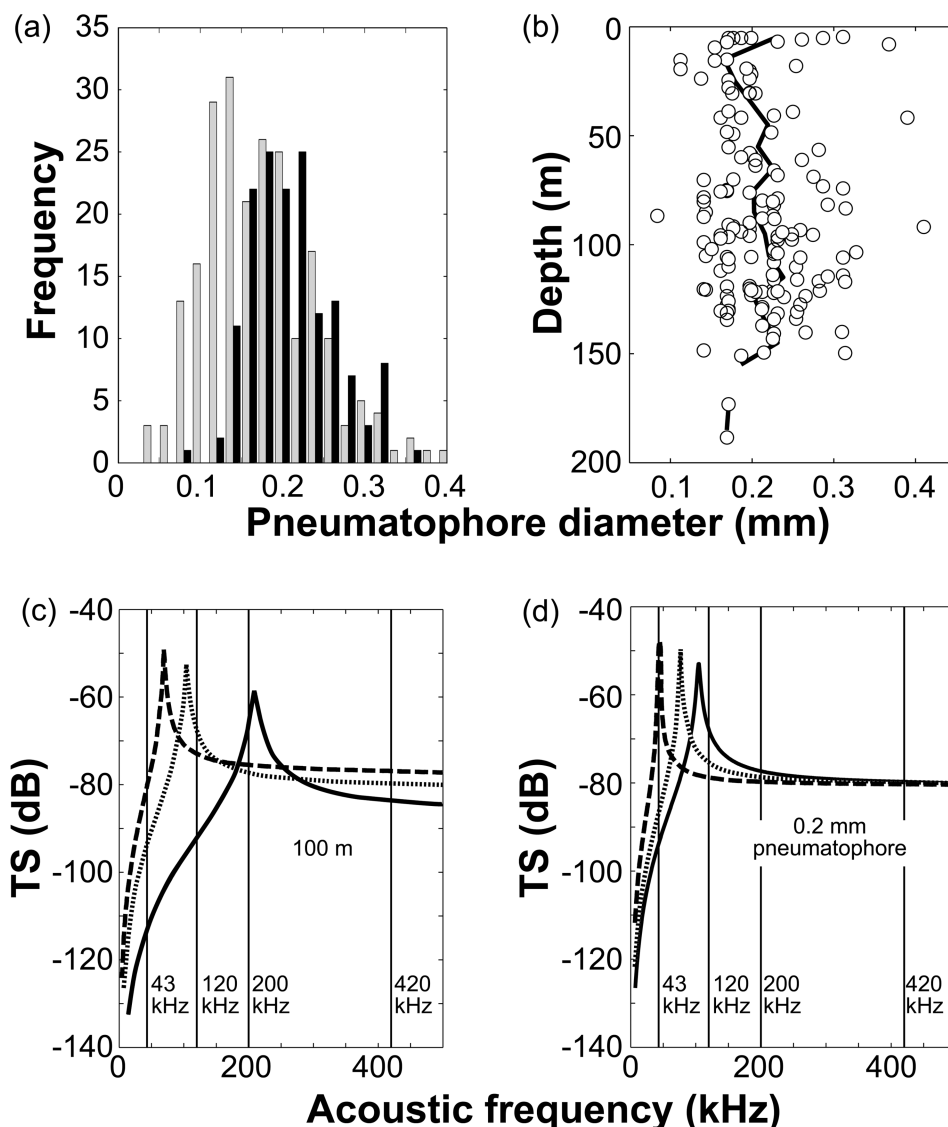
diameters of  $\sim 0.3$  mm were near resonance at 43 kHz, whereas pneumatophores near the mean diameter (0.21 mm) were close to resonance at 120 kHz, and small pneumatophores (0.1 mm) were within the resonance region at 200 kHz (Fig. 6c). For a 0.2 mm diameter pneumatophore, the resonance frequency increased while the scattering amplitude decreased with increasing depth as the hydrostatic pressure compressed the gas within the pneumatophore (Fig. 6d). Peak resonance occurred at  $\sim 10$ -m depth at 43 kHz and at  $\sim 100$ -m depth at 120 kHz (Fig. 6d). The distributions of TS values estimated from the scattering model for the measured pneumatophores detected by the image processor were narrow at the higher frequencies (200 and 420 kHz) and broader at the lower frequencies (43 and 120 kHz) where resonance was occurring. The mean and median TS values were lowest at 43 kHz (mean of  $-89.1$  dB, median of  $-90.2$  dB), highest at 120 kHz (mean of  $-72.0$  dB, median of  $-72.4$  dB), and both intermediate and similar at 200 kHz (mean of  $-77.2$  dB, median of  $-76.9$  dB) and 420 kHz (mean of  $-79.6$  dB, median of  $-79.8$  dB).

An examination of the acoustic record from BIOMAPER II coincident with the 4 h of manually examined videotape suggests that the presence of siphonulae at depth was associated with enhanced scattering at 120 kHz (Figs. 7, 8). In general, the presence of siphonulae in the video corresponded with regions of strong scattering, although the strongest scattering was not always coincident with the highest siphonulae densities. A zone of elevated scattering of approximately  $-60$  dB was generally present at 120 and 200 kHz within the

depth interval 50–150 m (Fig. 7). At the higher and lower frequencies, we observed consistent bands of elevated scattering that corresponded to the depth interval at which siphonulae were detected (Fig. 7), although at generally lower scattering levels. In addition, although elevated scattering at 43 kHz is indicated in the vicinity of the vehicle coincident with siphonulae, this scattering did not extend away from the vehicle and this is potentially an artifact that is currently under investigation. The estimated contributions by siphonulae to measured scattering at each of the four frequencies suggest that they can account for much of the observed scattering in regions where they are abundant (Fig. 8). Further, the shapes of the vertical distribution of predicted scattering from siphonulae and measured scattering from BIOMAPER II were generally similar at all frequencies (Fig. 8).

The MOCNESS tow taken in Jordan Basin revealed a mesozooplankton community that was numerically dominated by large and small copepods and ostracods (Fig. 9). Other taxa that are potentially strong scatterers such as thecosomate pteropods, euphausiids, and adult siphonophores were generally present in low densities. Small copepods dominated the zooplankton assemblage at the surface, whereas larger copepods (primarily *Calanus finmarchicus* and *Paraeuchaeta norvegica*) were abundant at depth (Fig. 9). When the sizes and densities of all taxa except siphonulae collected by the MOCNESS nets were used to predict the acoustic scattering profile (Fig. 9), the difference between the mean measured and predicted scattering strengths demonstrated a substantial

**Fig. 6.** (a) Size–frequency distributions of pneumatophore diameters from siphonulae larvae imaged by the video plankton recorder (solid bars) and recovered from the MOCNESS (shaded bars). (b) Scatterplot of pneumatophore diameter and depth for 154 siphonulae. The solid line indicates the mean diameter at 10-m intervals. (c) Theoretical scattering curves for 0.1 mm (solid line), 0.2 mm (dotted line), and 0.3 mm (dashed line) diameter siphonula pneumatophores at 100-m depth. (d) Theoretical scattering curves for a 0.2 mm diameter pneumatophore at 10-m (dashed line), 50-m (dotted line), and 100-m (solid line) depths.



underestimation of the mean measured scattering in the depth strata in which siphonulae occur. The discrepancy between the mean measured and predicted scattering strengths could have been due to the presence of siphonulae.

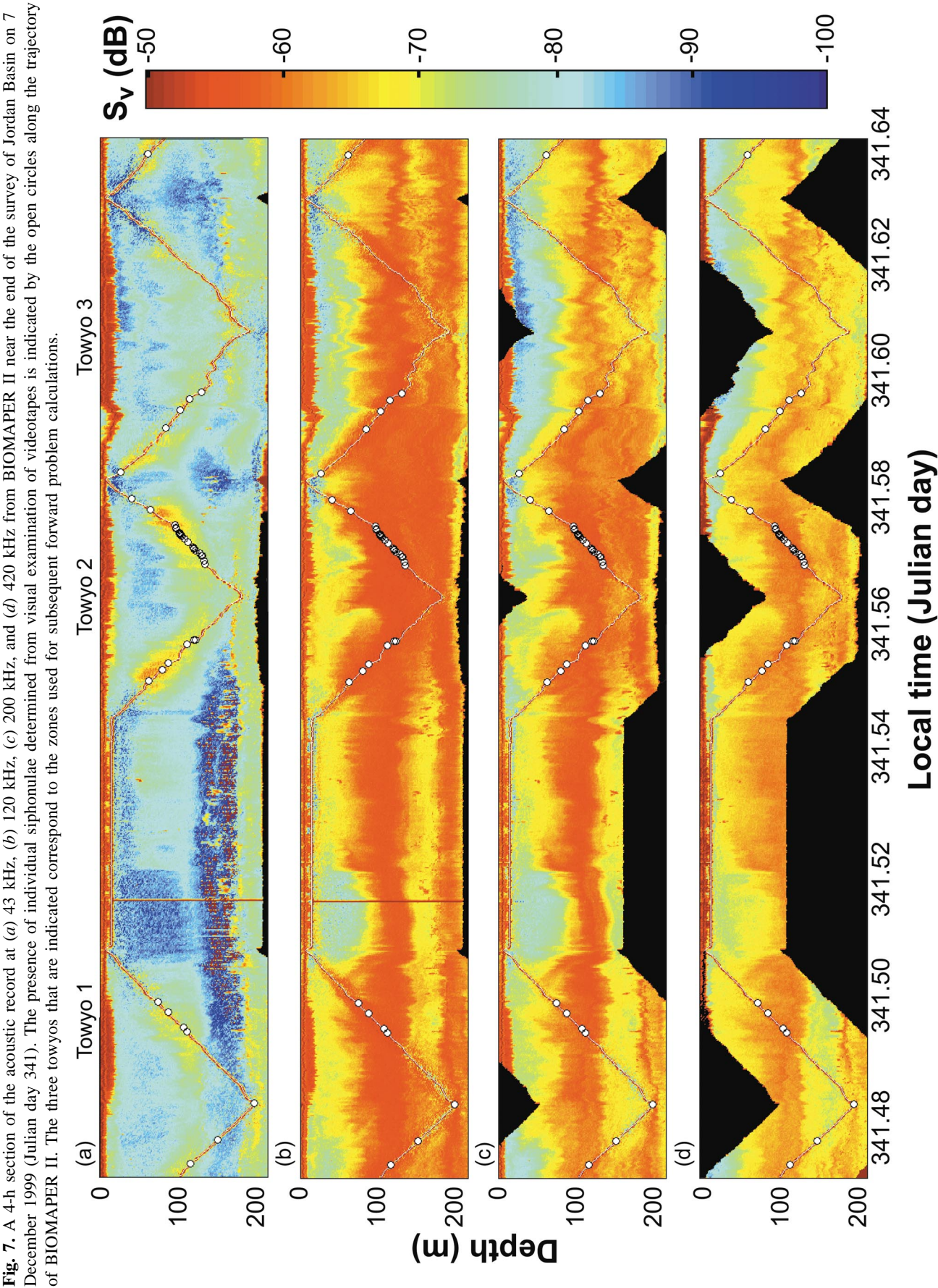
## Discussion

Siphonulae of *Nanomia bijuga* are able to secrete gas into their pneumatophore after 3–5 days of age (Sherlock and Robison 2000). The presence of a buoyant organ provides the siphonulae with potential control over their vertical distributions. Whether or not siphonulae are capable of depth regulation has not been determined (Sherlock and Robison 2000) and little is known about the vertical distributions of larvae. Bigelow (1911) reported that the larvae of three different genera including *Nanomia* (which had been classified

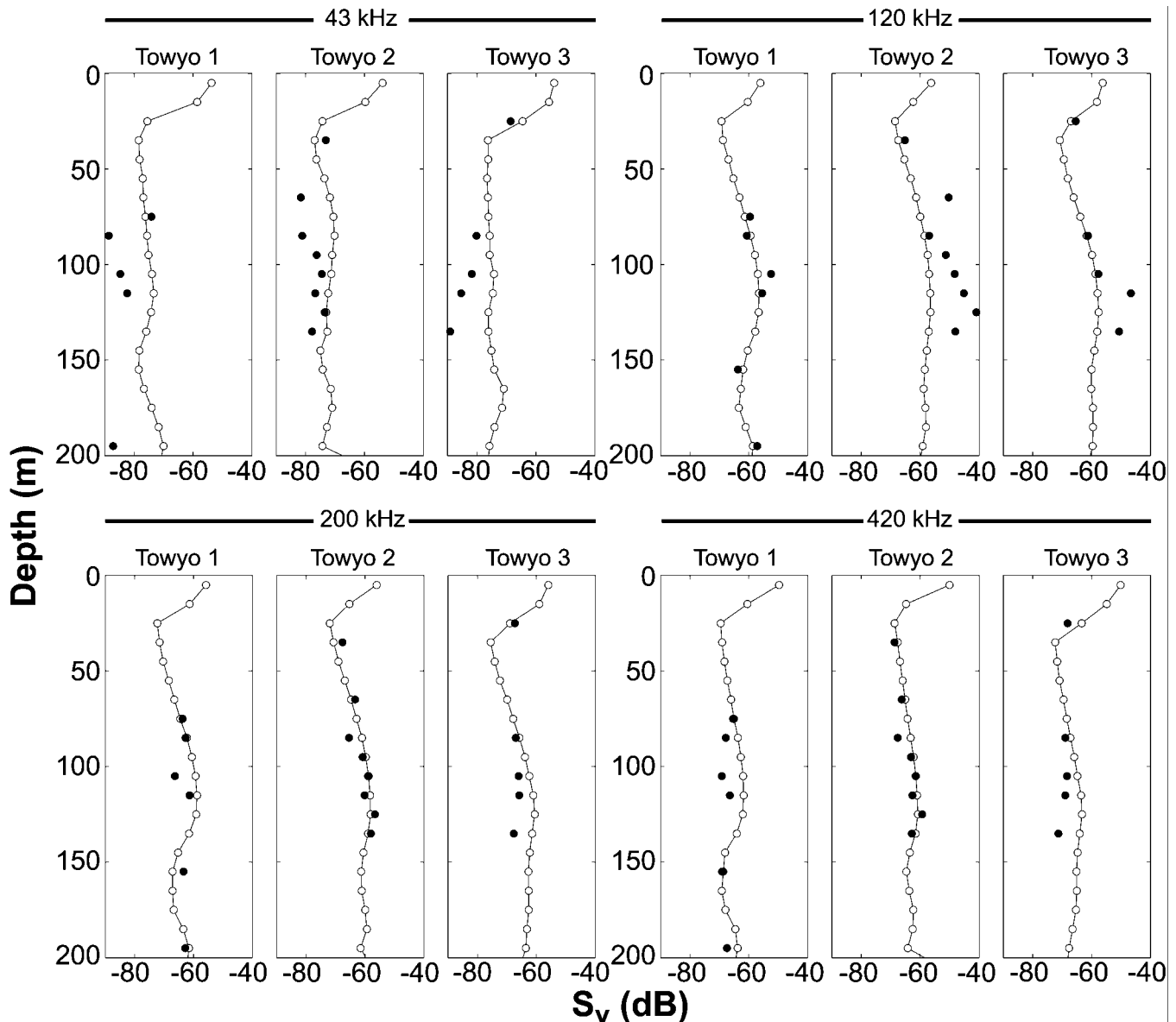
as *Physophora*) were taken at depths from 100–900 m during summer in the Mediterranean Sea. If the distributions that we observed in all three basins were a consequence of active depth regulation, then they suggest that siphonulae are capable of fairly precise control over their depths. Passive accumulation of siphonulae in density strata where they are neutrally buoyant is also possible; however, the range of densities within which siphonulae were most common varied among the three basins. It seems more likely that the siphonulae were distributed within favorable temperature strata and were able to modify their depth distributions to some extent.

Control over their vertical distribution provides siphonulae with the ability to regulate their thermal regime. Bigelow (1911) reported that temperature was the primary determinant of the vertical distributions of siphonophores. This may





**Fig. 8.** Mean measured volume backscattering strengths ( $S_v$ ) at 43, 120, 200, and 420 kHz, within 10-m depth intervals (○) in each of the three tow-yos demarcated in Fig. 7. The estimated volume scattering strengths (●) attributable to siphonulae are the product of siphonulae densities determined by the VPR in each depth stratum and the predicted backscattering cross section(s) of each of the pneumatophore(s) detected in each tow-yo stratum at each of the four frequencies.

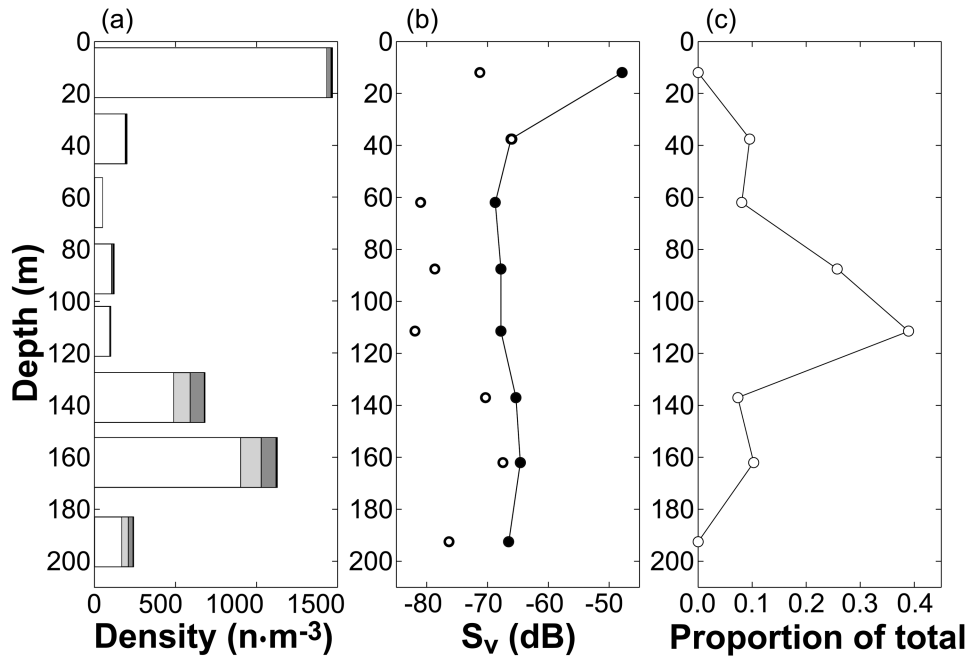


be important in optimizing conditions for growth and survival. Sherlock and Robison (2000) have demonstrated that the survival and growth of *N. bijuga* siphonulae was temperature dependent. It is interesting that the siphonulae that we observed tended to favor waters between 8 and 11°C. Survival of Sherlock and Robison's siphonulae was higher at 8°C than at 12 or 18°C, although their larvae were not fed and the lower temperature may have favored survival by reducing metabolic rates and slowing the rate of starvation. Although the diets of siphonulae are not well known, it is likely that they include microzooplankton prey items too small to be reliably detected or censused by our VPR and small mesozooplankton. Fluorescence was one potential indicator of areas of elevated chlorophyll and potentially elevated microzooplankton; however, siphonulae were not as-

sociated with regions of elevated fluorescence. Our surveys were conducted in December when primary production was already low and fluorescence may not have been well correlated with microzooplankton abundance. Although Sherlock and Robison (2000) demonstrated that fertilized eggs of *N. bijuga* were capable of developing into siphonulae in the absence of food, most siphonulae that we detected appeared to have a pair of extended tentacles suggesting that they were actively feeding on unknown prey. Based on the abundance of mesozooplankton and presumed abundance of microzooplankton in the Gulf of Maine during our survey, it is likely that the waters in which our siphonulae were distributed contained sufficiently abundant prey siphonulae.

The results from Wilkinson Basin suggest that siphonulae may exhibit a diel vertical migration pattern. Adult colonies

**Fig. 9.** (a) Numerical taxonomic composition of the MOCNESS tow in Jordan Basin conducted in the late evening of 6 December 1999. Each bar is placed at the center of the depth interval for the corresponding net. Small copepods ( $\leq 2.5$ -mm prosome length) are represented by open bars and large copepods ( $> 2.5$ -mm prosome length) are represented by lightly shaded bars. Ostracods are represented by darkly shaded bars, and other taxa are represented by solid bars. (b) The mean volume scattering strength for depth intervals that corresponded to the MOCNESS nets ( $\bullet$ ). We used the densities and sizes of each taxon in each of the MOCNESS nets to estimate the volume scattering that would be produced from such an assemblage. The predicted scattering for all taxa except siphonulae is indicated by the single points ( $\circ$ ). (c) The vertical distribution of siphonulae collected by the MOCNESS expressed as a fraction of the total number collected.



of *N. bijuga* have been reported to undertake diel vertical migrations off Italy (Barham 1963), and there was some indication that the distribution of the same species was shifted upward at night in Monterey Bay (Robison et al. 1998). The apparent diel vertical migration pattern that we observed in Wilkinson Basin was not evident in the other two basins. This may simply reflect behavioral plasticity of the siphonulae or an insufficiently large enough sample size to gain a complete picture of their vertical distributions. It is also possible that our inability to determine the species of siphonophores from VPR images has resulted in the pooling of larvae from two or more species that each exhibited different vertical distribution patterns. The taxonomic identities of our siphonulae are unknown because the VPR images do not provide sufficient detail to determine the genus or species. Identification of species from images alone is difficult, and Totton (1965) pointed out that siphonulae of different species of siphonophores appear very similar. We suspect that the siphonulae that we imaged may belong to the genus *Nanomia* because adult *N. cara* were present in the waters of the Gulf of Maine during our cruise. Rogers et al. (1978) reported that adult colonies of *N. cara* were abundant in the waters over the deep basins of the Gulf of Maine during the winter.

Siphonophores are believed to be able to maintain the diameter of their pneumatophore by secreting or absorbing carbon monoxide gas (Mackie et al. 1987). This also appears to be the case for siphonulae because the pneumatophore diameters did not appear to change with depth — a proxy

variable for hydrostatic pressure. If we assume a spherical pneumatophore geometry, then a 0.21 mm diameter pneumatophore at 10-m depth (2 atm) would contain 0.0084  $\mu\text{L}$  of gas. To maintain the same pneumatophore volume at 100 m (11 atm), a siphonula would need to secrete an additional 0.004  $\mu\text{L}$  of  $\text{CO}$  to restore the pneumatophore volume in 1 h after a downward migration of 100 m. Larimer and Ashby (1962) provided estimates of  $\text{CO}$  gas secretion rates for siphonophores of the species *Physalia physalis* ranging in pneumatophore volume from 25 to 120 mL. We are not aware of any estimates of the gas production rates of physonect genera such as *Nanomia*; however, a sigmoid curve predicting gas production as a function of pneumatophore volume provided a good fit to Larimer and Ashby's data for *P. physalis* (adjusted  $R^2 = 0.993$ ,  $p = 0.003$ ; our fit excluded one apparent outlier for a 14 mL pneumatophore). The gas production rate for a 0.21 mm diameter pneumatophore predicted using the fitted curve was 0.003  $\mu\text{L CO} \cdot \text{h}^{-1}$ , which agreed closely with our estimate of 0.004  $\mu\text{L CO} \cdot \text{h}^{-1}$ .

The gas-filled pneumatophore of adult siphonophore colonies is clearly a strong scatterer of certain frequencies of sound. Barham (1963) pointed out an apparent correlation between the depths of recorded deep scattering layers and concentrations of [physonect] siphonophores. Further, he noted that the gas bubble within the pneumatophores of *N. bijuga*, collected from an ascending scattering layer, was close to the resonance size at 12 kHz. These findings led Totton (1965) to speculate that siphonophores were the major causes of stratified zones of scattering throughout the oceans.

Stanton et al. (1994) demonstrated experimentally that freshly caught siphonophore pneumatophores produced strong echoes between 50 and 500 kHz in a laboratory tank (1 atm). Robison et al. (1998) used a 675-kHz sector-scanning sonar on an ROV to map the distributions of individual colonies in Monterey Bay. Their echogram revealed the presence of strong acoustic targets that were presumed to be adult individual colonies of *N. bijuga*. The median acoustical target strength of adult colonies of *N. cara* in the Gulf of Maine was -75 dB as measured with a dual-beam 420 kHz echosounder mounted on a 1-m<sup>2</sup> MOCNESS (Greene et al. 1998). More recently, Warren et al. (2001) mounted a pair of 24-kHz acoustic transducers on an ROV and recorded in situ TS values (mean of -62.5 dB) from adult colonies of *N. cara*. In this current study, it appears that the pneumatophores of siphonula larvae are also capable of producing relatively strong echoes because the dimensions of the small gas bubbles place them near, or within, the resonance zone at 43 kHz when the siphonulae are near the surface and at 120 kHz when they are near 100 m. The correlation between the presence of siphonulae and zones of enhanced backscatter in our echograms is consistent with our predictions that these small and otherwise inconspicuous organisms have a relatively strong acoustic signature that can account for much of the observed scattering in some depth strata.

Our forward problem calculations suggest that siphonulae present at our estimated densities could account for much of the observed scattering at all frequencies in the 4-h record from Jordan Basin. The observed and predicted patterns of scattering were derived from independent sources yet in several cases, both produced vertical scattering profiles that were similar in shape and frequently in magnitude. These forward calculations were highly sensitive to the pneumatophore diameters. Consequently, small shifts in the pneumatophore diameter could change the contribution to measured scattering by up to ~10 dB. At 120 kHz, our forward calculations substantially overestimated the measured scattering. The reasons for this overestimation are probably a combination of pneumatophore measurement errors and overestimation of their abundances. The pneumatophores on our VPR images were present as dark objects defined primarily by the reflection of the strobe on the surface of the pneumatophore. The relatively low resolution of the images combined with difficulty in determining the precise boundaries of the pneumatophore likely contributed to measurement error. Although we are confident that our overall estimates of the dimensions of the pneumatophores are reasonable, because measurements of preserved siphonula pneumatophores collected in a MOCNESS tow from Jordan Basin produced a similar mean diameter to the mean VPR-derived diameter, small errors in the dimensions of individual pneumatophores could drastically alter our prediction of acoustic scattering.

Our abundance estimates of the numbers of siphonulae per cubic metre were scaled up from relatively small volumes of water surveyed by the VPR. Consequently, small changes in the numbers of siphonulae imaged by the VPR could result in large changes in their abundance per cubic metre. Better estimates of both the pneumatophore dimensions and the abundances of siphonulae are certainly needed to improve our ability to predict individual components of observed scattering. However, the estimates of siphonula

densities from the VPR are supported by the correspondence between predicted and observed scattering at most frequencies, given the caveats mentioned earlier about the sensitivity of our predictions near 120 kHz. That our system is so sensitive to changes in the scattering properties of siphonulae is cause for both concern and optimism. Concern, because until we have survey systems capable of collecting quantitative, high-resolution images of larger volumes of water, we may not be able to predict the contribution by organisms such as siphonulae to measured scattering with better precision. We are also optimistic, in part because imaging systems capable of higher optical resolutions and larger sample volumes are being developed, but primarily because the sensitivity of scattering calculations to pneumatophore dimensions, pressure, and acoustical frequency means that it may well be possible to extract biologically meaningful information from our echograms given appropriate "sea-truth".

It is unlikely that the enhanced acoustical scattering in the vicinity of the siphonulae strata was the result of scattering from other zooplankton. The zooplankton composition of the water column in Jordan Basin appeared similar to that of the other basins with high densities of small copepods near the surface and larger copepods, primarily *Calanus finmarchicus*, below 150 m. Strong scatterers such as thecosomate pteropods, adult siphonophores, and euphausiids were not abundant at mid-depths and were generally distributed either near the surface or below 150 m. Thus, siphonulae shared the mid-depth region with relatively few other mesozooplankton potentially capable of producing strong acoustic returns. Other organisms such as larval fish may also contain a gas inclusion with similar acoustic properties to siphonophore pneumatophores. It is unlikely that fish larvae were responsible for the observed scattering during our study because reproductive activity of fishes in the Gulf of Maine is relatively less frequent during late fall and early winter compared with other times of the year (Sherman et al. 1987) and because fish larvae in our samples were associated with surface waters, whereas most siphonulae were concentrated at mid-depth. A MOCNESS tow collected at night on 7 December (day 341) in Jordan Basin contained few fish and those that were collected were only present near the surface at a density of ~1 m<sup>-3</sup>. The spatial separation of siphonulae from other targets with similar scattering properties suggests that acoustical frequencies near the resonance zone might be used to survey their distributions when a priori information confirming the absence of other gas-filled targets such as larval fish is available.

We recognize that our data set consists of a relatively small sample size of 175 siphonulae from a large area of the coastal ocean. Although our conclusions would certainly be more robust given a more complete picture of the distribution of these organisms, our VPR survey appears to be the only nonanecdotal description published thus far of the distribution of siphonulae. Our automatic image processing system was capable of detecting approximately 37% of individuals recorded by the VPR. This bias raises the question of how representative our distributional data are given that not all individuals that were present were actually detected. We are confident that the distributional patterns based on the image processor were representative of the true distributional pattern. Comparisons of siphonulae that were detected



by visual examinations with image processor detections revealed similar distributional patterns that differed only in the numbers of animals present in patches and the omission of sparse patches by the image processor. Advances in image processing software for the VPR are under development to improve identification (Tang et al. 1998) and extraction efficiency; however, even without such improvements, the VPR remains one of the few tools currently capable of documenting the fine-scale distributions of these enigmatic organisms.

In conclusion, this is the first published account of the in situ biology of physonect siphonulae. In this study, we have demonstrated significant evidence of the spatial and temporal variability and environmental conditions associated with siphonulae using an advanced oceanographic survey system that appears well suited for censusing fragile gelatinous organisms. There was evidence that the gas inclusion provided buoyancy control because siphonulae occupied relatively narrow depth strata and the pneumatophore diameter (and hence volume) appeared to be constant with depth. In addition, there was correlation between the presence of siphonulae and our measured acoustic data at all frequencies. This observation was consistent with theoretical predictions of scattering. Finally, the promise of acoustic detection of these small fragile organisms was demonstrated.

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## References

- Anderson, V.C. 1950. Sound scattering from a fluid sphere. *J. Acoust. Soc. Am.* **22**: 426–431.
- Austin, T.C., Arthur, R.I., Torkelson, T.C., Wiebe, P.H., and Stanton, T.K. 1998. BIOMAPER II: a towed bio-acoustic survey system for zooplankton and fish assessment. In *Proceedings of the Oceans Community Conference, 1998, Baltimore, Md.* Marine Technology Society, Columbia, Md. ([www.mtsociety.org/publications/index.cfm](http://www.mtsociety.org/publications/index.cfm)).
- Barham, E.G. 1963. Siphonophores and the deep scattering layer. *Science (Wash., D.C.)*, **140**: 826–828.
- Bigelow, H.B. 1911. The Siphonophorae. Reports of the scientific results of the expedition to the eastern tropical Pacific, in charge of Alexander Agassiz, by the U.S. Fish Commission steamer "Albatross", from October 1904 to March 1905, Lieut. Commander L.M. Garrett, U.S.N., commanding. *Mem. Mus. Comp. Zool. Harv.* **38**: 173–402.
- Davis, C.S., Gallagher, S.M., Berman, M.S., Haury, L.R., and Strickler, J.R. 1992. The Video Plankton Recorder (VPR): Design and initial results. *Adv. Limnol.* **36**: 67–81.
- Greene, C.H., Wiebe, P.H., Pershing, A.J., Gal, G., Popp, J.M., Copley, N.J., Austin, T.C., Bradley, A.M., Goldsborough, R.G., Dawson, J., Hendershott, R., and Kaartvedt, S. 1998. Assessing the distribution and abundance of zooplankton: a comparison of acoustic and net-sampling methods with D-BAD MOCNESS. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **45**: 1219–1237.
- Hamner, W.M., Madin, L.P., Alldredge, A.L., Gilmer, R.W., and Hamner, P.P. 1975. Underwater observations of gelatinous zooplankton: sampling problems, feeding biology, and behavior. *Limnol. Oceanogr.* **20**: 907–917.
- Larimer, J.L., and Ashby, E.A. 1962. Float gases, gas secretion and tissue respiration in the Portuguese man-of-war, *Physalia*. *J. Cell. Comp. Physiol.* **60**: 41–47.
- Mackie, G.O., Pugh, P.R., and Purcell, J.E. 1987. Siphonophore biology. *Adv. Mar. Biol.* **24**: 97–262.
- Medwin H., and Clay, C.S. 1998. *Fundamentals of acoustical oceanography*. Academic Press, San Diego, Calif.
- Morse, P.M., and Ingard, K.U. 1986. *Theoretical acoustics*. Princeton University Press, Princeton, N.J.
- Reeve, M.R. 1981. Large cod-end reservoirs as an aid to the live collection of delicate zooplankton. *Limnol. Oceanogr.* **26**: 577–579.
- Robison, B.H., Reisenbichler, K.R., Sherlock, R.E., Silguero, J.M.B., and Chavez, F.P. 1998. Seasonal abundance of the siphonophore, *Nanomia bijuga*, in Monterey Bay. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **45**: 1741–1751.
- Rogers, C.A., Biggs, D.C., and Cooper, R.A. 1978. Aggregation of the siphonophore *Nanomia cara* in the Gulf of Maine: observations from a submersible. *Fish. Bull.* **76**: 281–284.
- Sherlock, R.E., and Robison, B.H. 2000. Effects of temperature on the development and survival of *Nanomia bijuga* (Hydrozoa, Siphonophora). *Invertebr. Biol.* **199**: 379–385.
- Sherman, K., Smith, W.G., Green, J.R., Cohen, E.B., Berman, M.S., Marti, K.A., and Goulet, J.R. 1987. Zooplankton production and the fisheries of the Northeastern Shelf. In *Georges Bank. Edited by R.H. Backus*. MIT Press, Cambridge, Mass. pp. 268–282.
- Stanton, T.K., Wiebe, P.H., Chu, D., Benfield, M.C., Scanlon, L., Martin, L., and Eastwood, R.L. 1994. On acoustic estimates of zooplankton biomass. *ICES J. Mar. Sci.* **51**: 505–512.
- Stanton, T.K., Chu, D., Wiebe, P.H., Martin, L.V., and Eastwood, R.L. 1998a. Sound scattering by several zooplankton groups. I. Experimental determination of dominant scattering mechanisms. *J. Acoust. Soc. Am.* **103**: 225–235.
- Stanton, T.K., Chu, D., and Wiebe, P.H. 1998b. Sound scattering by several zooplankton groups. II. Scattering models. *J. Acoust. Soc. Am.* **103**: 236–253.
- Tang, X., Stewart, W.K., Huang, H., Gallagher, S.M., Davis, C.S., Vincent, L., and Marra, M. 1998. Automatic plankton image recognition. *Artif. Intell. Rev.* **12**: 177–199.
- Totton, A.K. 1965. A synopsis of the Siphonophora. *British Museum (Natural History)*, London.
- U.S. GLOBEC. 1992. Northwest Atlantic implementation plan. U.S. GLOBEC Program, Report No. 6, June 1992. U.S. GLOBEC Program, Leonardtown, Md. ([www.usglobec.org/reports/reports.pdf.files/rep6.pdf](http://www.usglobec.org/reports/reports.pdf.files/rep6.pdf)). pp. 1–81.
- Warren, J.D. 2001. Estimating Gulf of Maine zooplankton distributions using multiple frequency acoustic, video and environmental data. Ph.D. thesis, Massachusetts Institute of Technology, Woods Hole Oceanographic Institution, Boston, Mass. Thesis No. 2001-03.
- Warren, J.D., Stanton, T.K., Benfield, M.C., Wiebe, P.H., Chu, D., and Sutor, M. 2001. In situ measurements of acoustic target strengths of gas-bearing siphonophores. *ICES J. Mar. Sci.* **58**: 740–749.

- Wiebe, P.H., and Benfield, M.C. 2001. Zooplankton sampling with nets and trawls. In *Encyclopedia of ocean sciences*. Edited by J.H. Steele, K.K. Turekian, and S.A. Thorpe. Academic Press, London. pp. 3237–3253.
- Wiebe, P.H., and Benfield, M.C. 2003. From the Hensen net toward four-dimensional biological oceanography. *Prog. Oceanogr.* **56**: 7–136.
- Wiebe, P.H., Stanton, T.K., Benfield, M.C., Mountain, D., and Greene, C.H. 1997. High-frequency acoustic volume backscattering in the Georges Bank coastal region and its interpretation using scattering models. *IEEE J. Oceanic Eng.* **22**: 445–463.
- Wiebe, P.H., Stanton, T.K., Greene, C.H., Benfield, M.C., Sosik, H.M., Austin, T., Warren, J.D., and Hammar, T. 2002. BIOMAPER II: an integrated instrument platform for coupled biological and physical measurements in coastal and oceanic regimes. *IEEE J. Oceanic Eng.* **27**: 700–716.

## Appendix A. Estimation of pneumatophore target strength

The target strength (TS) is defined in terms of  $f_{\text{pneumatophore}}$  by the relationship  $TS = 10 \log_{10} |f_{\text{pneumatophore}}|^2 = 10 \log_{10} \sigma_{\text{bs}}$ . The units of TS are dB relative to  $1 \text{ m}^2$ , and  $\sigma_{\text{bs}}$  ( $\text{m}^2$ ) is the backscattering cross section. In fact,  $f_{\text{pneumatophore}}$  is a complex function of the pneumatophore radius, the acoustic frequency, the density contrast ( $g = \frac{\rho_{\text{pneumatophore}}}{\rho_{\text{water}}}$ ) and the sound

velocity contrast ( $h = \frac{c_{\text{pneumatophore}}}{c_{\text{water}}}$ ) between the gas in the pneumatophore and the surrounding water.

The influence of the linearly increasing water pressure with depth, which has a direct influence on the density and sound speed contrasts between the gas in the pneumatophore and the surrounding water, was included in the scattering model using the following approach (Medwin and Clay 1998). The depth dependence of the ambient water pressure is given by  $p_z = p_0 + \rho_0 g z \approx (1 + 0.1z) \text{ atm}$ , where  $p_0$  is the sea level atmospheric pressure ( $p_0 = 1 \text{ atm}$ ),  $g$  is the acceleration due to gravity ( $9.8 \text{ m}\cdot\text{s}^{-2}$ ),  $\rho_0$  is the water density ( $1030 \text{ kg}\cdot\text{m}^{-3}$ ),

which was assumed constant throughout the water column, and  $z$  is the depth. It was assumed that the pressure,  $p_{\text{pneumatophore}}$ , of the gas in the pneumatophore had to equal the external water pressure (ignoring effects due to surface tension) and that the gas in the pneumatophore was an ideal gas at constant temperature. In addition, as is discussed in greater detail in the main text, there was no apparent depth dependence to the radii of the measured pneumatophores. As a result, the volume of gas ( $V$ , which contains  $n$  moles of gas of molecular weight  $M$ ) in the pneumatophore was assumed constant as a function of depth. This observation of constant pneumatophore volume, combined with the assumption of an ideal gas at constant temperature ( $T$ ), implies that the density of the gas increases in an amount proportional to the increase in the water pressure (ideal gas law:  $p_{\text{pneumatophore}} V = nRT$ , which is equivalent to  $p_{\text{pneumatophore}} = \rho_{\text{pneumatophore}} \frac{RT}{M}$ , where  $R$  = universal gas constant =  $MR$ , and  $R$  is the gas constant for air). Thus, the density contrast between the pneumatophore gas and the surrounding water at depth  $z$  was estimated by  $g = g_0(1 + 0.1z)$ , where  $g_0 = 0.0012$  is the density contrast between the pneumatophore and the surrounding water at the surface (Warren 2001). Although the pneumatophore is filled with  $\text{CO}_2$ , the properties of air were used as an approximation.

The effect on the sound speed contrast resulting from the increasing water pressure with depth can also be estimated. For an ideal gas (Morse and Ingard 1986, p. 228) at constant volume and temperature, the sound speed of the gas in the pneumatophore is equal to the square root of the pressure

over the density,  $c_{\text{pneumatophore}} = \sqrt{\frac{p_{\text{pneumatophore}}}{\rho_{\text{pneumatophore}}}}$ . Because the

pressure is proportional to the density at all depths, the sound speed in the gas is constant. With the additional assumption that the sound speed variation of the water is small as a function of depth, it can be concluded that, to first order, the variation in the sound speed contrast,  $h$ , with depth is small. Consequently,  $h$  was taken to be constant throughout the water column, using a value of 0.22 (again, for air) taken from Warren (2001).