A continuous, underway fish egg sampler

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ABSTRACT

We describe a method to sample the highly contagious distribution of pelagic fish eggs. CUFES, the continuous, underway fish egg sampler, consists of a submersible pump, concentrator, electronics and sample collector. This system operates continuously and under nearly all sea conditions, providing a real-time estimate of the volumetric abundance of pelagic fish eggs at pump depth, usually 3 m. CUFES-derived estimates of volumetric abundance agree well with those from nets towed at pump depth and with areal abundance estimated from vertically integrated plankton tows. CUFES has been used successfully to sample the eggs of menhaden, pinfish, sardine, and anchovy off the coasts of the eastern and western United States and South Africa. Two large patches of eggs of the Atlantic menhaden were sampled off North Carolina in winter 1993–94, had a linear scale of 5–10 km, and were found in waters between the Gulf Stream and mid-shelf front. Spawning location may be related to bathymetry. CUFES is now being used to estimate spawner biomass by the daily egg production method. An optical plankton counter provided accurate estimates of the number of Atlantic menhaden eggs sample by CUFES.

INTRODUCTION

Pelagic eggs of fish are highly aggregated in time and space. Such aggregation affects both the ecology of fish and our ability to study and manage them. In particular, estimates of egg abundance in time and space are used to estimate the spawning biomass of populations of pelagic fish and are thus needed to understand the status and dynamics of fish populations in order to best manage them.

Contagious distributions are difficult to sample. Conventional methods, nets towed at discrete stations, are limited in their accuracy, precision and sensitivity, and are labour intensive and hence costly. In addition, such methods require the dedicated use of a ship, are limited by adverse conditions, and produce samples which await analysis ashore. We therefore sought an alternative method to sample fish eggs. We wished to sample continuously, underway, for nearly all conditions at sea, and with other operations able to be performed simultaneously. We sought a real-time assessment of egg abundance, to facilitate adaptive sampling (Thompson, 1992) and minimize subsequent laboratory work. Here we describe our system and its successful sampling of the Atlantic menhaden, *Brevoortia tyrannus*, pinfish, *Lagodon rhomboides*, northern anchovy, *Engraulis mordax*, and the Pacific sardine, *Sardinops sagax*.

The impetus to develop this system was a desire to investigate the hypothesis that the Atlantic menhaden spawns during storms at the western wall of the Gulf Stream (Checkley *et al.*, 1988). Subsequent development occurred as part of the South Atlantic Bight Recruitment Experiment, SABRE, to investigate recruitment variation in estuarine-dependent fish, including the Atlantic menhaden. Most recently, CUFES has been used to assess spawning stocks of sardine and anchovy off California and South Africa.

Pelagic eggs of fish are often spawned at night by adults in schools, resulting in distributions of eggs

that are highly contagious. The fertilized eggs are buoyant and typically hatch in 1–4 days. Although the depth of spawning varies within and between species, the distribution of eggs appears to achieve a surface maximum early in the development of the egg (Ware and Lambert, 1985; Pommeranz and Moser, 1987; Tanaka, 1992; Cambalik, 1993). Turbulence will mix buoyant eggs downward but they will remain most abundant at or near the surface (Pommeranz and Moser, 1987; Sundby, 1991; Tanaka, 1992; Cambalik, 1993). Hence, we felt it appropriate to sample near the surface to allow inference about areal abundance. We hypothesized that the concentration of eggs at a single depth is significantly related to areal abundance.

Initially, we tested an optical plankton counter (OPC, Herman, 1988) to sense eggs of Atlantic menhaden in the ambient plankton. We hypothesized that pelagic fish eggs, due to their relatively large size, would, when abundant, constitute a statistically identifiable peak in the particle size spectrum assessed optically by the OPC. It was discovered, however, that menhaden eggs are optically similar to other, numerically dominant plankters (copepods) of approximately half their diameter, owing to the relative transparency of menhaden eggs compared with other plankters. Similar results have subsequently been reported for the eggs of Baltic cod, *Gadus morhua* (Wieland and Köester, 1996). Hence, we sought to increase the 'signal-to-noise' ratio for sensing pelagic fish eggs by concentrating them relative to physically smaller, although optically indistinguishable, particles. To this end, we used a scaled-up version of the zooplankton concentrator of Herman *et al.* (1984).

We wished to sample as large a volume as reasonable so as to maximize our sensitivity to detect eggs in low abundance, at the edge of a patch of recently spawned eggs. To do so, we used a high-volume, submersible pump having a flow rate comparable to that of the towed, *in situ* OPC.

Our system, named the continuous, underway fish egg sampler (CUFES) therefore consists of a high-volume, submersible pump fixed rigidly to the ship's hull and, on deck, devices to concentrate egg-sized particles and to collect sequential samples of these particles on a mesh. Other, electronic instruments may be used to analyse particles in the flow between the concentrator and the sample collector. Here, we present results from use of the OPC; video has been combined with the OPC but will be discussed elsewhere. It is important to recognize that, to date, the routine use of CUFES requires the visual examination of collected samples. However, our OPC results indi-

cate that automated, electronic counting of eggs is feasible.

Other systems are analogous to CUFES in part but not entirely. Lenz *et al.* (1995) describe a video-recording system for *in situ* studies of ichthyoplankton. Miller and Judkins (1981) and Star and Mullin (1981) describe plankton pumping systems. Murdoch *et al.* (1990) provide a method of shipboard identification and counting of pelagic fish eggs but it does not address sample collection and requires the use of photography and is thus not real-time. None of these contains a concentrating mechanism nor means of real-time counting of eggs.

Here we discuss deployment of our system in the winter of 1993–94, on the NOAA ship *Oregon II* off North Carolina, and in spring 1996, on the NOAA ship *David Starr Jordan* off Southern California. Our goals were twofold. First, to test CUFES, including comparisons with conventional methods. Second, to survey the regions of most probable spawning of the Atlantic menhaden, northern anchovy, and Pacific sardine in order to assess the distribution and abundance of their eggs. Our intent in this paper is not to present comprehensive results of either egg surveys or patch studies but, rather, to demonstrate the potential for using this method to attain these larger objectives. We describe our underway, egg sampling system; present results for the menhaden, sardine, and anchovy; and discuss our system's efficacy for censusing the pelagic eggs of fish. We conclude that our system samples fish eggs well and enables improved assessment of their distribution and abundance.

MATERIALS AND METHODS

CUFES

Our pumping system consists of three elements (Fig. 1): first, an *in situ*, submersible pump fixed to the ship's hull; second, a device to concentrate large particles, including fish eggs; and third, analysis devices, including a laboratory OPC, video system, and mechanical sample collector (MSC). These elements enable us to: (i) pump ≈ 0.5–1.0 m^3 min^{-1} from a nominal depth, 3 m; (ii) concentrate large particles in a flow of 15–20 l min^{-1}; and (iii) electronically sense and physically collect particles for real-time assessment of the concentration of the target particle type, e.g. eggs of the Atlantic menhaden or Pacific sardine, under nearly all sea conditions.

Ship requirements include: (i) a location to mount the submersible pump, preferably amidships; (ii) a laboratory or protected area for the concentrator and

Figure 1. Continuous underway fish egg sampler, CUFES. System details may vary between installations but always include submersible pump, concentrator and mechanical sample collector. Pump depth is nominally at 3-m depth. See text for details.
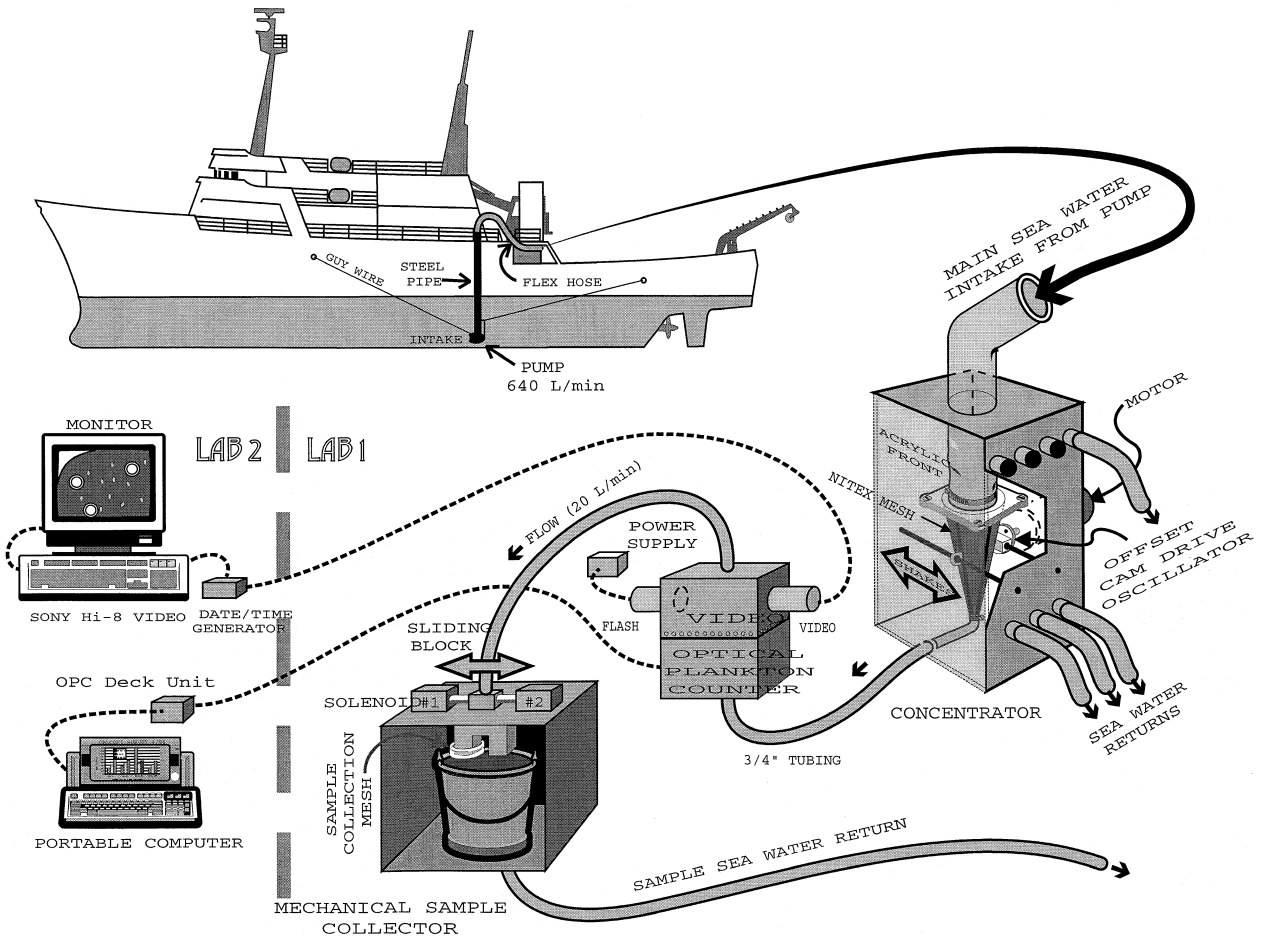
analysis equipment, including associated hoses; and (iii) electrical power for the submersible pump. The total weight of CUFES is ≈800 lbs (365 kg). CUFES installation requires ≈2 days and removal ≈1 day.

Our pump is a semi-vortex pump (Ebara model 80 DVSU 62.22), a type previously shown to not greatly damage zooplankters (Omori, 1985; Checkley *et al.*, 1992). Electrical power is either 440 V AC and 4.2 amp or 220 V AC and 8.4 amp. The pump is mounted on the lower end of a 6.4-m (21-foot) long, 10-cm (4-inch) i.d., schedule 80 (0.85-cm (0.337-inch) wall thickness) steel pipe which is orientated vertically and held by clamps secured by bolts or welds to the ship's gunwale and hull just above the water line. In addition, the vertical pipe is guyed from near the pump, underwater, to ship structures fore and aft on deck. Water from the pump enters a flexible, PVC-reinforced, 7.6-cm (3-inch) i.d. pressure hose which, in turn, enters the vertical pipe near the pump and

exits through the upper end of the pipe and thence to the concentrator. A 440 or 220 V AC power line is attached to the pipe and energizes the pump. The pipe is faired below the water line to reduce drag. The pipe and attached pump can swing between the vertical and horizontal positions, to enable maintenance and transport out of the water when not in service. The pump depth may be adjusted between the surface and 3 m by raising and lowering the vertical pipe.

The concentrator consists of an oscillating Nitex net (570 cm^2 total area) submerged in a 50-litre vessel, and is adapted from a smaller device (Herman *et al.*, 1984). Water enters the concentrator from the top and exits either as filtrate (≈97% of flow) through its side, after passing through the net, or as concentrate (≈3% of flow) at the bottom end of the net and vessel. The Nitex is sewn tightly within a metal frame. The frame with Nitex moves laterally over a 2.3-cm range at 5.75 Hz. The mesh size of the Nitex is selected to

maximize the efficiency of retention of eggs of the target species and simultaneously minimize retention of optically similar, though physically smaller, particles, and clogging. In general, we use a mesh size ≈1/3–2/3 the equivalent spherical diameter of the target egg. For eggs of the Atlantic menhaden (sphere, ≈1.4–1.8 mm diameter), we use 1-mm Nitex; for eggs of the northern anchovy (spherical ellipsoid, 1.2–1.4 mm major axis, 0.65–0.82 mm minor axis) and co-occurring Pacific sardine (sphere, 1.4–2.1 mm diameter), we use 0.500 mm Nitex. The filtrate is directed overboard through flexible hoses, one of which has a ball valve (not shown in Fig. 1) to control water level in the concentrator.

Water from the concentrator flows by gravity at ≈20 l min^{-1} in 1.9-cm (0.75-inch) i.d. clear, flexible tubing to electronic sensors and finally into the mechanical sample collector. Fish eggs may be distinguished from other particles by use of the OPC, based on optical size, and video, based on other characteristics (shape, dimensions). Particles, including eggs, are then collected in the MSC on a Nitex mesh. The MSC is a solenoid-driven, computer-controlled device which directs flow from the 1.9-cm (0.75-inch) clear, flexible tubing coming from the concentrator to one side or the other of a knife-edge flow separator and thence onto a removable, Nitex filter, analogous to the cod end of a plankton net. The flow is redirected periodically from one side, hence filter, to the other. The filtrate is directed overboard while, upon completion of the sample, the retained material is immediately inspected, and the target eggs counted, under a dissecting microscope and then fixed and preserved in a small bottle, e.g. scintillation vial of 20 ml, with buffered, 5% formalin for later analysis.

The concentrator net eventually becomes clogged. The pump and oscillator motor are then turned off, and the Nitex mesh in the concentrator is cleaned in place using a brush and by spraying with water under pressure. The period between clogging depends on the flow into the concentrator, the concentrator mesh size, and the particulate matter in the sampled water. Our experience is that, with full flow (≈640 l min^{-1}), the concentrator mesh must be cleaned every 2 to 12 h. Cleaning time is ≈5–10 min. Occasionally, other, large particles (salps, drift kelp) enter the system and necessitate it being shut down and cleaned.

Periodically, the system's mean flow rate is measured. The flow from each discharge hose and the MSC is measured individually. These data are combined to estimate flow into the concentrator and from the concentrator to the MSC.

Concurrent with pumping, data are logged periodically for date, time, position, and variables such as temperature, salinity, and chlorophyll *a* fluorescence. The former three variables are from a GPS. The latter measurements are at times made using our pumped flow and, at other times, the ship's seawater flow, the former being preferred. In either case, we assume these measurements are comparable to those of CUFES in time and location.

On the January 1994 cruise of the NOAA vessel *Oregon II*, for which data are presented, a Turner Designs fluorometer was placed in line between the concentrator and the MSC and its output voltage recorded. This fluorometer was calibrated in the laboratory with pure chl *a* and the resulting factor used to estimate *in vivo* chl *a* concentration in the pump flow. Temperature and salinity were measured in the ship's seawater flow using Seabird Electronics temperature and conductivity sensors. Position data were from the ship's GPS. Data reported here were recorded at the end of each CUFES sample interval, either 5, 10, or 15 min.

Net comparisons

On several cruises, net collections were made simultaneous to pumping to compare (a) retention of eggs by CUFES relative to nets towed at the same depth and (b) estimates of the concentration of eggs on volumetric (eggs m^{-3} at 3 m, CUFES) and areal (eggs m^{-2}, vertically towed net) bases.

Retention efficiency was studied by collecting plankton at pump depth with either a bongo net (McGowan and Brown, 1966), MOCNESS (Wiebe *et al.*, 1976), or Tucker (1951) trawl. In the former case, a 70-cm bongo with paired 333-μm-mesh nets was lowered rapidly to pump depth; a new CUFES collection begun; after a known period, usually ≈5 min, sampling ended by rapid retrieval of the net and changing CUFES samples. In the latter cases, either a 1-m^2 MOCNESS or 1-m^2 Tucker trawl with 333-μm-mesh nets was lowered to pump depth and successive nets opened and closed at the same time a sample was collected from CUFES. In all cases, flowmeters were used to measure the volume of water filtered. Ship speed varied from 0.2 to 1.5 m s^{-1} (1–3 kt (knots)) and tow duration 2–5 min.

Areal distribution comparisons were made with a CalVET net and standard protocols (Smith *et al.*, 1985). The CalVET net was raised at 1 m s^{-1} from 70 m or bottom, if less. CalVET and CUFES samples were taken simultaneously, on opposite sides of the vessel. The CUFES sampling period (≈5 min) bracketed the CalVET ascent (≈1-min duration). Each pair

of collections was made at a different station and hence under different atmospheric and oceanographic conditions, although the latter were not monitored.

Laboratory analysis

Ashore, all eggs of the respective target species of fish were enumerated in each sample. Identification keys were Fahay (1983) for Atlantic menhaden and pinfish, Moser and Ahlstrom (1985) for northern anchovy, and Ahlstrom (1943) for Pacific sardine. Eggs of the Pacific sardine were assigned to 11 developmental stages (Lo *et al.*, 1996). Data on eggs collected per unit time were combined with the pump flow rate to estimate concentration (E_{CUFES} (eggs m^{-3})). Data on eggs collected per net tow were combined with flow meter data to calculate egg concentration (E_{net} (eggs m^{-3})).

Optical plankton counter

The OPC was used both at sea, off North Carolina, and in the laboratory. At sea, a laboratory OPC was in line between the concentrator and MSC (Fig. 1). Data were acquired with a DOS computer with custom software and synchronized to GPS. In the laboratory, individual particles were introduced into the OPC flow. A captive population of Atlantic menhaden was induced to spawn (Hettler, 1981). The fertilized eggs and resultant larvae were maintained in filtered sea water at 18°C in a 12:12 light:dark cycle. Periodically, eggs and yolk-sac larvae were removed and passed through the OPC in the laboratory. In addition, we also used live eggs made opaque by staining with methylene blue, eggs preserved in a 5% solution of formalin in sea water, and plastic beads the size of eggs.

RESULTS

General operation

CUFES is a robust system. It has operated at ship speeds to 12 kt ($\approx$6 m s^{-1}). We have used it successfully in all conditions encountered to date, ranging from calm to storms (22 m s^{-1} winds, 4-m seas). In heavy seas on a rolling ship, the system draws in pulses of bubbles from breaking waves. This does not affect fish egg sampling but can affect particle detection by electronic sensors. A bubble trap reduces the number of bubbles passing through the OPC and other instruments; subsequent filtering of the data during analysis largely eliminates this problem.

CUFES has operated successfully for four winters (1993–1997) off North Carolina in studies of the Atlantic menhaden, two springs (1996) off California

in studies of Pacific sardine and northern anchovy, and in autumn 1996 off South Africa (not discussed here), sampling pilchard, *Sardinops sagax*. Sampling depth was 3 m. The flow rate into the concentrator varied between 610 and 680 l min^{-1}, with a mean value of 640 l min^{-1}. This rate appears unaffected by clogging of the concentrator net, its mesh size (0.5 or 1.0 mm), ship motion, or other conditions.

It is of interest to know the approximate region sampled by the pump. At rest, water is drawn from a volume surrounding the pump intake (8-cm diameter). As the ship moves at rate S, the pump, intake forward, draws water (F, flow rate) from a nominal cylinder the diameter (ϕ) of which decreases with increasing speed. For F of 640 l min^{-1}, the ship speed for which ϕ and the pump intake are the same diameter is 2.1 m s^{-1} ($\approx$4 kt). At 5 m s^{-1} ($\approx$10 kt), ϕ is 5.2 cm. Eggs do not respond behaviourally to the flow field at the pump intake and hence avoidance is not an issue. Other zooplankters, e.g. copepods, are capable of avoidance yet should be well sampled because their escape speeds ($\approx$10 body lengths s^{-1}, 20 mm s^{-1} for a 2-mm long copepod) are far less than the speed at the pump intake.

Egg retention

Four sets of comparisons were made between CUFES and towed nets to investigate retention efficiency. Two hypotheses were tested. First, we used an ANOVA to test whether towed nets and CUFES provide equivalent estimates of fish egg concentration. Second, to test the null hypothesis that fish egg abundances estimated by towed net and CUFES are not related, we used Pearson's correlation coefficient, r, and parameter estimates for the linear regression between these variables. These tests were made with egg concentration (E, eggs m^{-3}) both untransformed (E) and transformed ($\ln(E + 1)$) to account for inhomogeneity of variances.

To estimate retention efficiency, collections were made from the NOAA ship *Oregon II* off North Carolina in winter 1993–94. CUFES was equipped with 1-mm-mesh Nitex in its concentrator, as the target species was Atlantic menhaden (egg diameter $\approx$1.6 mm). The nets were of 333-μm-mesh Nitex. In the first comparison, a patch of pinfish was discovered and sampled with CUFES and bongo nets. Estimates of the concentration of pinfish eggs by CUFES and bongo nets were highly correlated (Table 1 and Fig. 2), although an ANOVA indicated a significant effect of gear type ($F_{1,11} = 43.13$, $P < 0.001$). In the second case, a patch of eggs of the Atlantic menhaden was sampled with CUFES and a 1-m^2 MOCNESS. Eight paired

Table 1. Retention of eggs of pinfish, *Lagodon rhomboides*, and Atlantic menhaden, *Brevoortia tyrannus*, by CUFES and towed nets deployed at 3-m depth. Eggs of both species are spherical. Concentration of eggs (eggs m^{-3}) is from CUFES (E_{CUFES}) and towed net (E_{net}). See text for details. Values in parentheses are 95% confidence limits of parameter estimates. * indicates slope significantly different from one and n.s. a slope not significantly different from one or an intercept not significantly different from zero. Number of observations 10 for *L. rhomboides* and 13 for *B. tyrannus*.

Egg Species	Diameter (mm)	Mesh (mm) Net	CUFES	Least-squares linear regression Untransformed Equation	r^2	Transformed Equation	r^2
Lagodon rhomboides	~1.0	0.333	1.000	E_{CUFES} = 0.25 E_{net} + 0.46 (0.16—0.34) (−3.24—4.16) * n.s.	0.83	ln(E_{CUFES} + 1) = 0.65 ln(E_{net} + 1) − 0.039 (0.23—1.08) (−1.52—1.44) n.s. n.s.	0.61
Brevoortia tyrannus	~1.6	0.333	1.000	E_{CUFES} = 0.76 E_{net} + 0.38 (0.50—1.01) (−1.7—2.50) n.s. n.s.	0.80	ln(E_{CUFES} + 1) = 0.82 ln(E_{net} + 1) + 0.19 (0.66—0.99) (−0.13—0.51) * n.s.	0.92

Figure 2. Volumetric abundances of egg concentration measured using CUFES and towed nets. Linear regression of ln-transformed, volume-based abundances from CUFES (E_{CUFES}, eggs m^{-3}) and towed nets (E_{net}, eggs m^{-3}) shown for eggs of the Atlantic menhaden and pinfish. See text for details and Table 1 for regression statistics.
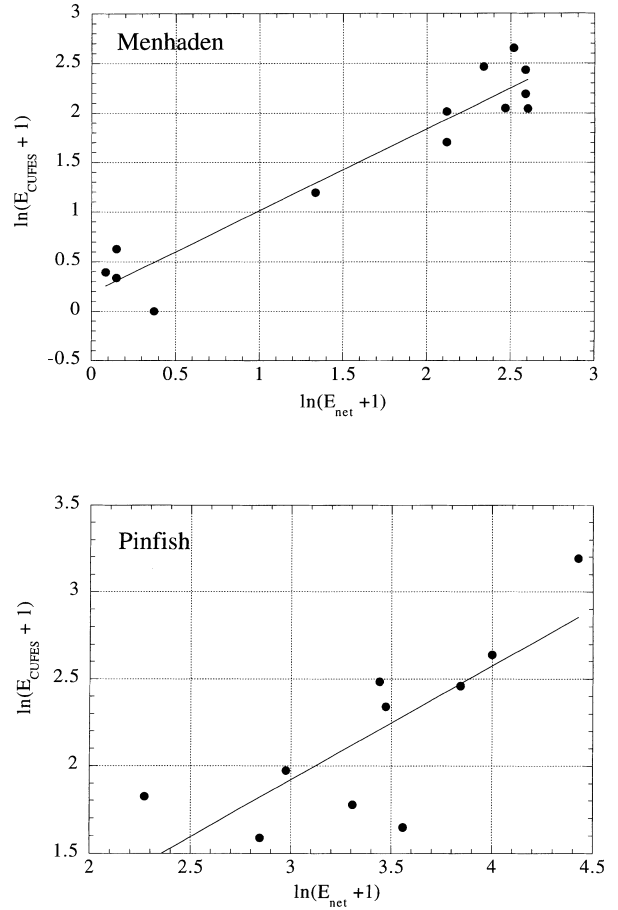
collections were made. An ANOVA showed no significant effect of gear type on egg concentration ($F_{1,14}$ = 2.64, P = 0.126). In the third case, two MOCNESS collections were made between the surface and 5 m simultaneous with pump samples taken at 3 m. The ANOVA indicated no significant effect of gear type ($F_{1,6}$ = 0.268, P = 0.623). In the fourth case, a patch of Atlantic menhaden eggs was sampled four times using CUFES and a Tucker trawl. Once again, ANOVA indicated no effect of gear type ($F_{1,6}$ = 1.29, P = 0.299). Data for Atlantic menhaden have been combined in Table 1 and Fig. 2; net- and CUFES-derived estimates of egg abundance are highly correlated (r^2 = 0.80 and 0.92 for untransformed and ln-transformed data, respectively). The slopes of the linear regressions for the untransformed data indicate

Table 2. Abundance of eggs of northern anchovy, *Engraulis mordax*, and Pacific sardine, *Sardinops sagax*, estimated using CUFES and CalVET nets. CUFES sampled at 3-m depth. Eggs of the Pacific sardine are spheres and those of the northern anchovy are oblate spheroids. Concentration of eggs (eggs m^{-3}) is from CUFES (E_{CUFES}) and CalVET net (E_{CalVET}). See text for details. Values in parentheses are 95% confidence limits of parameter estimates. * indicates slope or intercept significantly different from zero, n.s. indicates no such difference. Number of observations is 91 in each case. In 88 of these, the CalVET was towed from 70 m to the surface and in those cases, areal abundance (eggs m^{-2}) is 3.5 times volumetric abundance (eggs m^{-3}).

Egg		Mesh (mm)		Least-squares linear regression			
				Untransformed		Transformed	
Species	Diameter or major × minor axes (mm)	CalVET	CUFES	Equation	r^2	Equation	r^2
Engraulis mordax	~1.4 × 0.8	0.150	0.500	$E_{CUFES} = 0.52\,E_{CalVET} + 0.015$ (0.47—0.59) * (−0.079—0.109) n.s.	0.75	$\ln(E_{CUFES} + 1) = 0.75\,\ln(E_{CalVET} + 1) - 0.022$ (0.68—0.82) * (−0.051—0.007) n.s.	0.85
Sardinops sagax	~1.8	0.150	0.500	$E_{CUFES} = 5.8\,E_{CalVET} - 2.3$ (4.0—7.6) * (−7.7—3.1) n.s.	0.31	$\ln(E_{CUFES} + 1) = 1.3\,\ln(E_{CalVET} + 1) + 0.30$ (1.1—1.5) * (0.09—0.51) *	0.62

retention of eggs by CUFES relative to towed nets to have been 0.76 ± 0.25 (mean ± SD(mean)) for the Atlantic menhaden and 0.25 ± 0.09 for pinfish.

CUFES–CalVET comparisons

Comparative collections were made using CUFES and CalVET nets during cruise JD9603 of the NOAA ship *David Starr Jordan* off Southern California in March 1996 (Table 2, Fig. 3). We tested the same pair of hypotheses and tests as in the previous section. Our null hypothesis of no relation between volumetric concentration of eggs estimated using the two methods was rejected for both northern anchovy and Pacific sardine for both untransformed ($F_{1,89} = 267$, $P < 0.0001$, and $F_{1,89} = 40.9$, $P < 0.0001$, respectively) and transformed (ln([eggs] + 1)) data ($F_{1,89} = 504$, $P < 0.0001$, and $F_{1,89} = 143$, $P < 0.0001$). Areal abundance (eggs m^{-2}) is 3.5 times the CalVET volu-

Figure 3. Comparison of egg abundance from CUFES (E_{CUFES}, eggs m^{-3}) sampling at 3 m and vertically integrated haul of CalVET (eggs m^{-3}) for northern anchovy and Pacific sardine. See text for details and Table 3 for regression statistics.
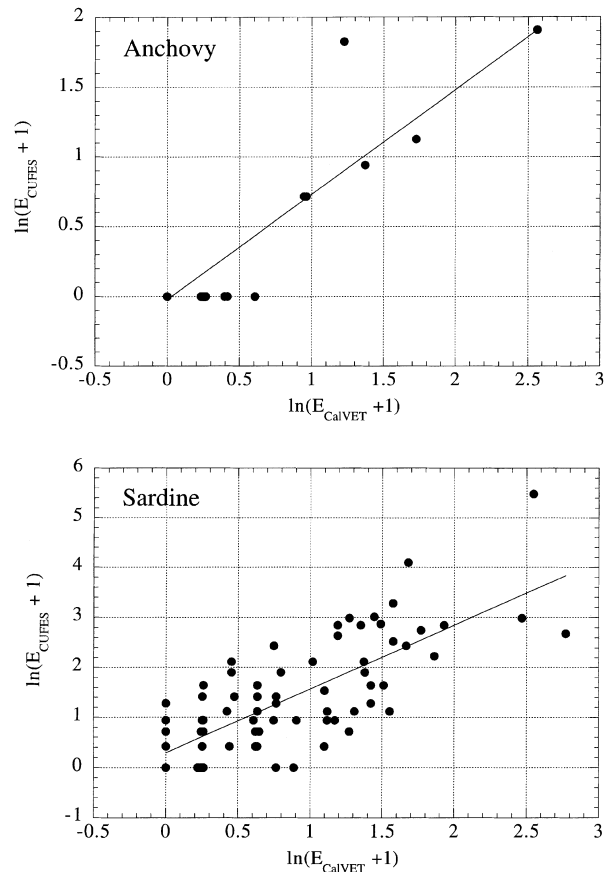
Figure 4. Comparison of CUFES and CalVET catches of Pacific sardine eggs of different developmental stage during cruise JD9603. The ratio of the sum of eggs collected by CUFES to the sum of eggs collected by CUFES and CalVET nets is shown in relation to developmental stage of egg. The sum of all eggs collected is shown in parentheses for each stage. Stage one eggs (shaded) are not used in DEPM (Lo et al., 1996).
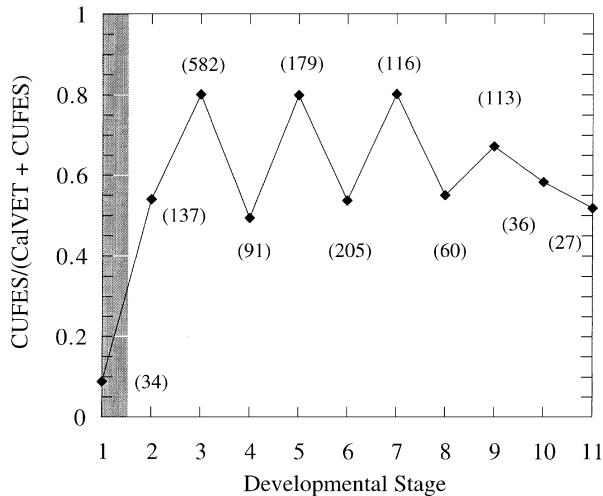
metric abundance (E_{net}, eggs m^{-3}) for most (88 of 91) of the collections in this study (those from 70 m to the surface). For each developmental stage, the sum of eggs collected by CUFES was divided by the sum of eggs collected by CUFES and CalVET nets. This ratio was unrelated to developmental stage ($r^2 = 0.09$; Fig. 4).

Mapping

Data were collected on 15–20 January 1994, during cruise 9308 of the NOAA ship *Oregon II* near the Gulf Stream in Onslow Bay (Fig. 5). Data for the distribution and abundance of Atlantic menhaden eggs and water temperature, salinity, and chl *a* concentration, are given in Fig. 6. The ship steamed at about 10 kt (≈ 5 m s^{-1}). The CUFES sample interval varied between 5 and 15 min (mean, 11 min), corresponding to ≈ 1.5–9 km (mean, 3 km) and 21–63 m^3 (mean, 46 m^3). A total of 545 samples was obtained over 103 h and 1480 km.

The Gulf Stream Front (GSF, Checkley *et al.*, 1988), with temperature 18–21°C and salinity ≈ 36.35, occurred from the SW to the NE, with cooler, less saline water to the west and north. A Gulf Stream filament appeared to enclose a tongue of cooler and less saline shelf water which extended east. High salinity ($\geq \approx 36.35$) and chl *a*-rich water occurred just north of the GSF and to the south of the cold tongue.

Chl *a* was also maximal to the north-east. Atlantic menhaden eggs occurred in two distinct patches (Fig. 6), each of 5–10 km scale. The first was at the GSF and the second in slightly cooler water to the west. Both patches were at locations near Cape Lookout with a bottom depth of 20–30 m. The number of eggs in a sample ranged from 0 ($n = 461$) to 595 in one 5- min collection.

The concentration of menhaden eggs has been summed within bins of temperature, salinity, and chl *a* and compared with sampling effort to indicate under what conditions eggs were most often collected (Fig. 7). The modal temperature for egg occurrence was 17.5°C and range 15–21°C. The modal salinity was 36.26 and range 36.10–36.45. Eggs were found over a broad range of chl *a* concentrations but, in general, not less than 0.1 μg chl *a* l^{-1}.

Optical plankton counter

We sought to test our original hypothesis that eggs, when abundant and concentrated by CUFES, would be manifest in the OPC size distribution. To this end, for two transects (cases A and B) through the westernmost patch with abundant menhaden eggs, we compared the size-frequency distribution of particles sensed by the OPC in the patch with that for a nearby control area, further along each transect, with few eggs (Fig. 8). OPC data and CUFES samples were acquired over 20 min. The OPC data were binned by digital size and size-frequency distributions constructed. For each bin, the control data were subtracted from the patch data. A peak, corresponding to the menhaden eggs, is evident in the differenced data in both cases. Integration beneath these peaks and between their approximate limits in digital size (22–59 digital size units, dsu) yields estimates of 534 and 1066 particles per 20 min for the two cases A and B, respectively. The numbers of eggs collected were 548 and 998, respectively. Hence, the OPC-derived estimate of egg-sized particles was 0.98 and 1.1 times the true number of eggs collected for cases A and B. A Gaussian model was fitted to the differenced histogram data for 22–59 dsu. The mean and SE was 40 ± 1 dsu for case A and 38 ± 1 dsu for case B.

The response of the OPC to introduced particles is shown in Table 3. Digital size (dsu) for eggs of the Atlantic menhaden increased from 44, one day post fertilization (dpf), to nearly 81 at 3 dpf, immediately prior to hatching. OPC response declined upon hatching and increased thereafter during development of the yolk-sac larva. The equivalent spherical diameter (*ESD*), estimated from the regression of Herman *et al.* (1991) for opaque particles, ranged from ≈ 0.7 to 1.0 mm for eggs and yolk-sac larvae. Stained, live eggs

Figure 5. Site map, bathymetry, and cruise track from cruise 9308 of NOAA vessel *Oregon II,* January 1994. Cruise track begins at eastern edge and ends to the west. Shown are locations at end of each CUFES sample interval. See text for details.
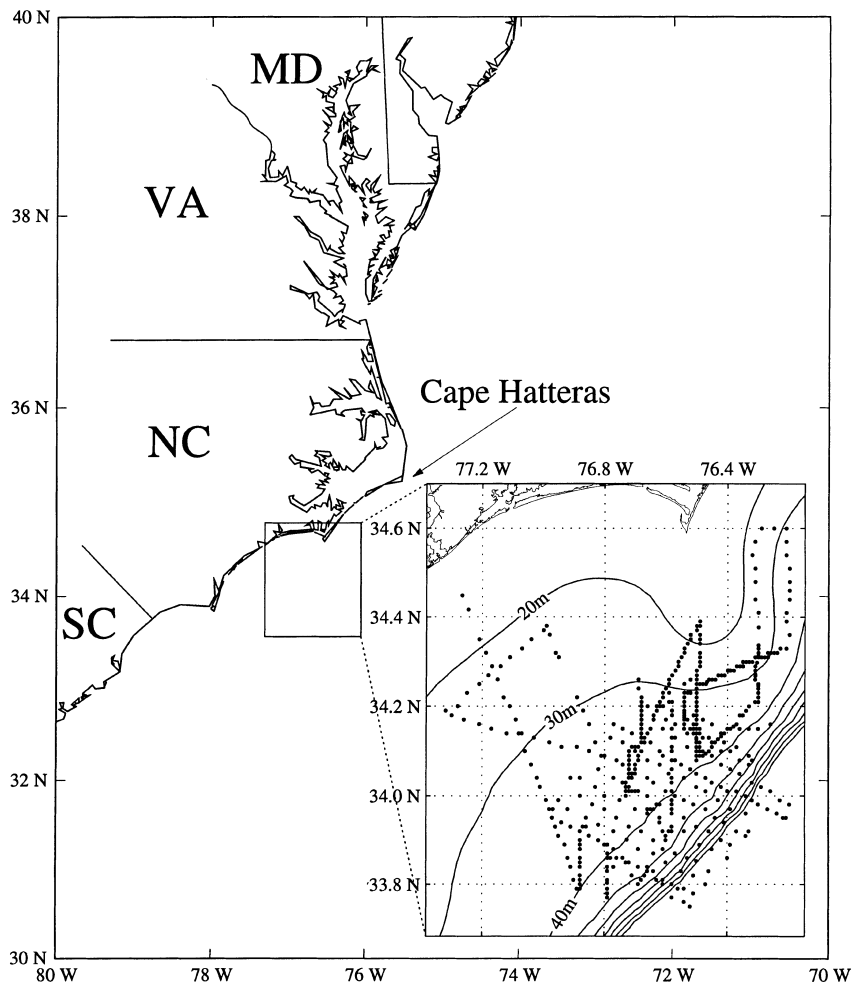

and opaque, plastic beads the size of menhaden eggs had *ESD*s of 1.5–1.6 mm, while formalin-preserved eggs were of intermediate value.

DISCUSSION

CUFES has proven to be a reliable system with which to sample pelagic eggs of fish. It provides shipboard data on the distribution and abundance of target species of eggs under virtually all sea conditions in which we have deployed it thus far. Importantly, these include conditions under which conventional samplers, including nets, may not be able to be used due to high winds and heavy seas. CUFES attributes include the following. (1) Sampling is continuous under nearly all sea conditions. This results in far more water sampled while at sea and no loss of samples in adverse conditions or 'dead time' while in transit between stations.

(2) The rate at which water is sampled is constant. In our use of CUFES reported here, the filtration a rate has been ≈640 l min^{-1}, independent of conditions, e.g. rate of ship movement and sea state. Flow rate may vary between installations, depending on the hydraulic head (height from sea to concentrator) and wall friction of the hose (distance from pump to concentrator). (3) Sampling can occur simultaneous with other ship activities, underway or on station. For example, acoustic surveys of juvenile and adult fish can be concurrent with sampling of eggs. (4) Fish eggs are quantitatively sampled. (5) CUFES samples are more easily analysed than those from conventional samplers, such as CalVET nets, owing to their concentration by relatively large-mesh Nitex used in the concentrator and sample collector, and due to the action of the concentrator. (6) Continuous sampling yields data series amenable to temporal and spatial analysis.

Figure 6. CUFES and related data from cruise 9308 of NOAA vessel *Oregon II*, January 1994. All measurements are for 3 m depth. See text for measurement details. Maps were made by using data at locations shown in Fig. 5 to construct a uniform grid, which was then colour contoured.
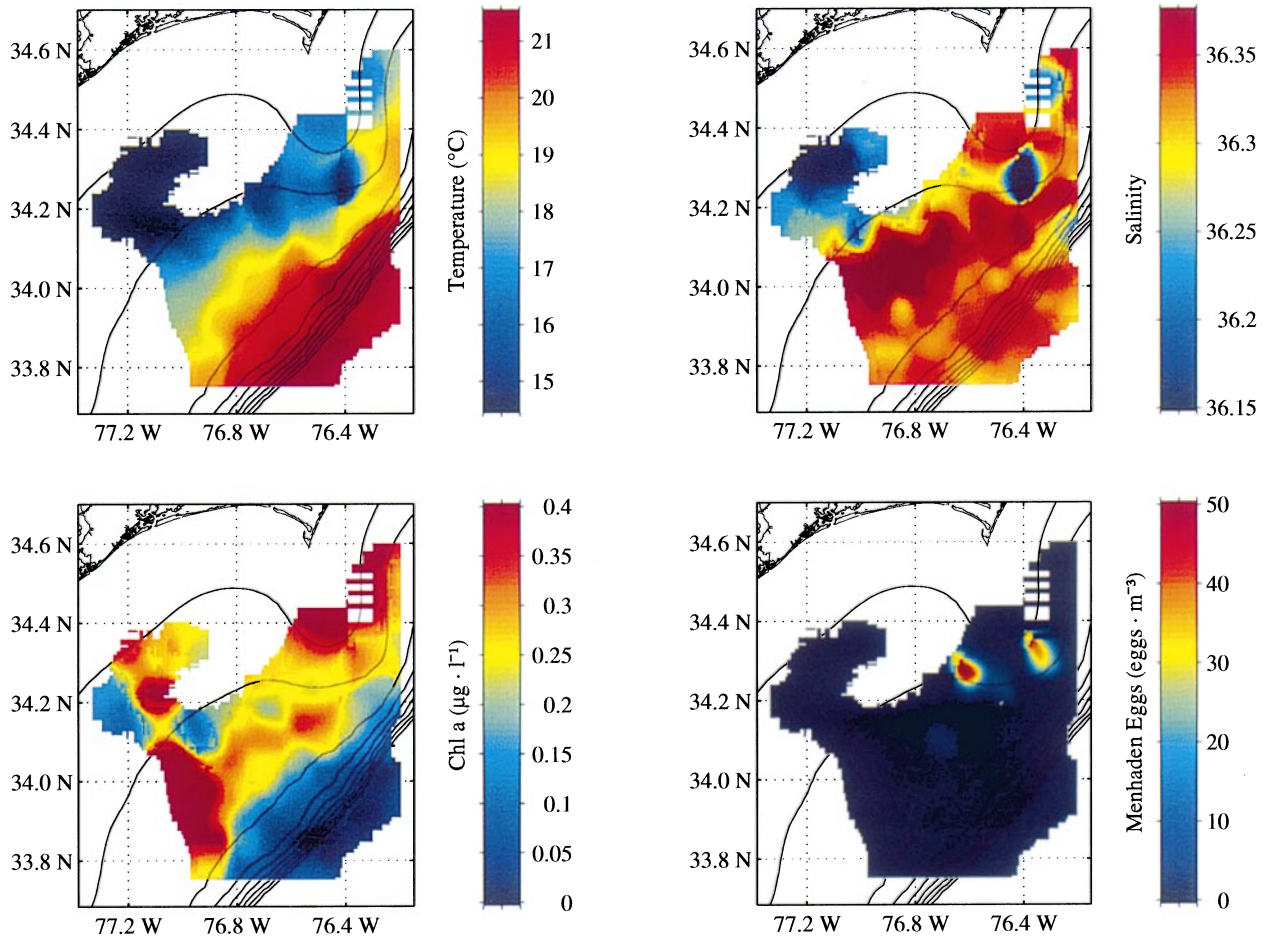

The usefulness of data from CUFES depends on the degree to which they compare with conventional samples. On both volumetric (Table 1) and areal (Table 2) bases, CUFES compares favourably with nets. Concentrations of eggs of pinfish and Atlantic menhaden at 3-m depth estimated from MOCNESS, Tucker, or bongo collections and CUFES were highly correlated (r^2, 0.61–0.92). Pinfish eggs are ≈ 1.0 mm-diameter spheres and some extrusion was expected through the 1-mm Nitex concentrator net and appears to have occurred (Table 1); the slope of the linear regression of E_{CUFES} on E_{net} was 0.25, significantly less than unity, and the ANOVA indicated an effect of sampling on egg abundance. Atlantic menhaden eggs are ≈1.6 mm-diameter spheres; the slope of the analogous regression did not differ significantly from unity, nor did an ANOVA indicate an effect of gear type on egg abundance. More data, hence statistical power,

may show CUFES, with a 1-mm concentrator mesh, to retain menhaden eggs with an efficiency less than unity. Regardless, it is the consistency of this value that is important. CUFES has provided representative estimates of volume-based egg abundance for both species for which this comparison has been made.

Our data indicate that eggs 1.6 times the mesh dimension are retained completely by CUFES, whereas eggs with a diameter equal to that of the mesh dimension are retained with only a 26% efficiency. Lower retention of eggs by CUFES than nets may be due to several processes. First, chance alone. Second, extrusion of eggs through the concentrator mesh. Third, disintegration due to mechanical damage. The yolk in eggs of menhaden and sardine, which normally possess a large perivitelline space early in development, is often dispersed through the entire egg volume after having passed through CUFES, as if the vitelline

Figure 7.   Histograms of frequency of occurrence of all samples (filled circles in Fig. 5; open bars) and menhaden egg occurrence (filled bars), in relation to temperature, salinity, and chl *a*. All measured at 3 m.
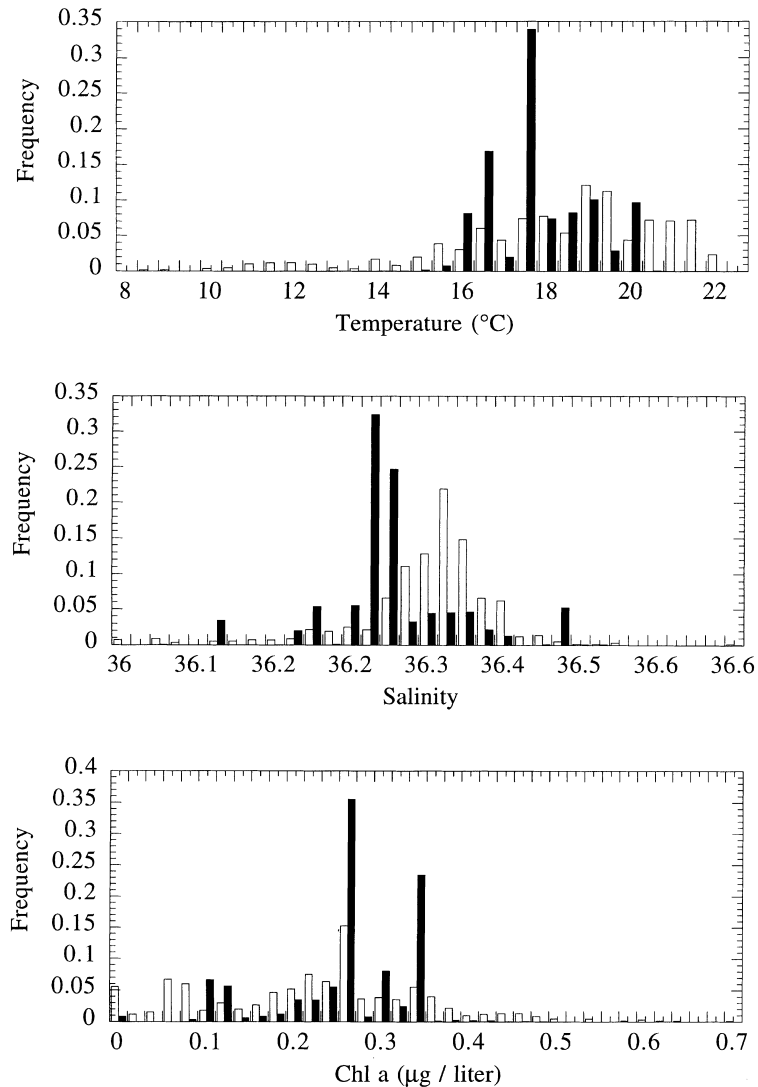

membrane were ruptured but the chorion remained intact. Rarely, empty egg membranes are found. It is not known at present where such damage arises, although we suspect it is in the mechanical sample collector. Video recordings show eggs passing between the concentrator and mechanical sample collector to be in good condition, with yolk mass intact (Fig. 9). Effort is underway to correct this damage by use of a more gently-acting cod end with a large filtering section immersed in water. Even eggs with dispersed yolk are able to be assigned a stage of development, however.

The correlation between the concentration of pelagic fish eggs estimated using CUFES sampling at 3 m and nets towed horizontally at 3 m (Table 1, Fig. 2)

and vertically over the range of eggs (Table 2, Fig. 3) is consistent with variability known for replicate collections of the plankton (Winsor and Clarke, 1940), including fish eggs (Silliman, 1946). Smith and Hewitt (1985) repeated collections of anchovy eggs with a CalVET net every 12 min for 84 min at 60 stations off southern California. Correlation coefficients for collections 12 min apart increased from a median value of 0.74 (range, W:0.25–0.93) for eggs 1 day old to 0.94 (W:0.91–0.97) for eggs 3 days old. Correlation (r^2) of abundance (pteropods m^{-3}) estimated from right and left bongo nets in oblique hauls ($n = 20$) was 0.96 for *Limacina inflata* and 0.66 for *Cavolinia inflexa* (calculated from Table 3 of McGowan and Brown, 1966). Each collection device, including towed nets and

Figure 8. Optical plankton counter (OPC) data from CUFES. Two cases (A, B) are shown. For each, OPC data were collected for 20 min inside a patch (upper panels) and 20 min immediately outside a patch (middle panels) in a control region. The difference between the patch and control size-frequency distributions is shown in the lower panels. Note the peak, with mode ≈ 38 digital size units, corresponding to menhaden eggs.
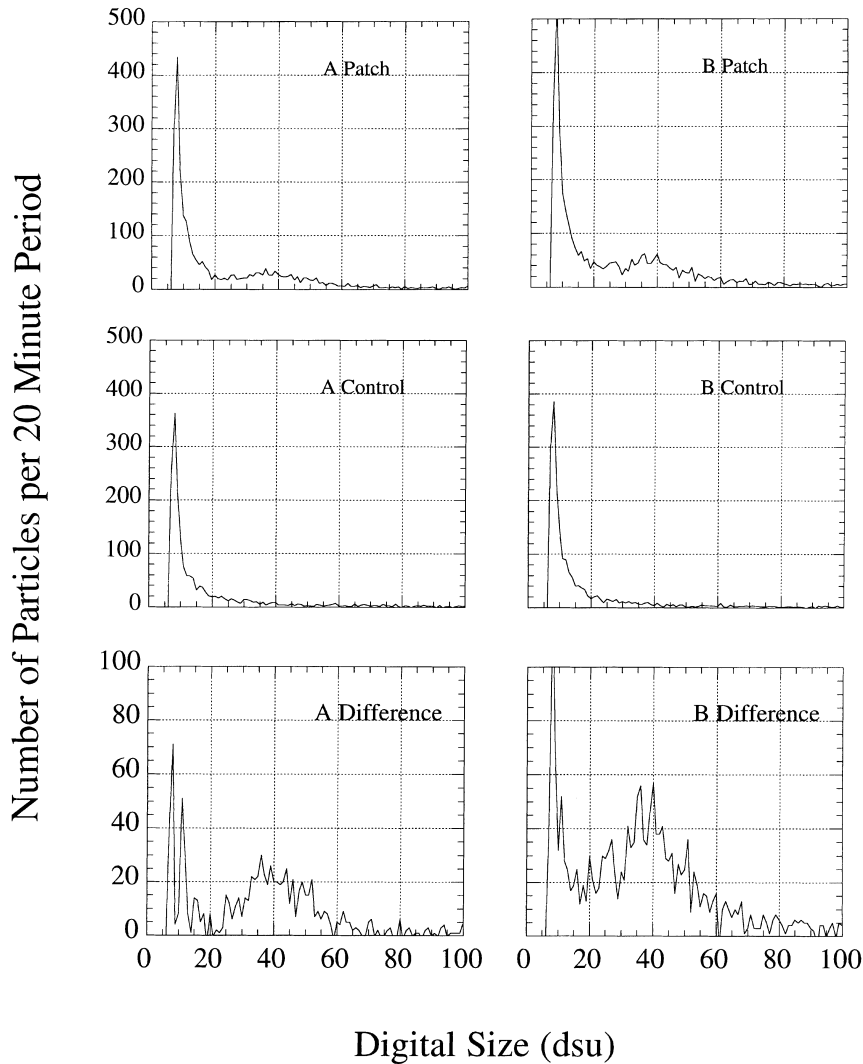

CUFES, integrates over some volume of water and hence scale of patchiness, resulting in variable abundance estimates. Sources of variation in the horizontal include spawning in aggregations, and accumulation of eggs in physical features such as fronts and Langmuir convergences, and sources of variation in the vertical include depth of spawning, ascent rate of eggs, and physical mixing and stratification. Ideally, one might sample with CUFES at the 'median depth' (*sensu* Ware and Lambert, 1985), whether directly measured (from stratified collections with a pump or nets), predicted from a model (Cambalik, 1993), or determined using the average of past measurements. The median depth will vary (1–6 m for Atlantic mackerel, Ware and Lambert, 1985; 3–9 m for the northern anchovy, Pommeranz and Moser, 1987; see also Sundby, 1991). We have opted to sample at a constant depth, usually 3 m, most simple and robust logistically, and use the results of a model (Cambalik, 1993) and empirical comparisions (Table 2, Fig. 3) using CUFES and CalVET, to infer areal abundance.

The use of CUFES to estimate the abundance of eggs in a patch or area depends on the relation between the volumetric abundance of eggs estimated with CUFES and the areal abundance estimated by nets towed vertically over the range of occurrence of

Figure 9. Digitized images of menhaden eggs in CUFES acquired with OPC/video. Diameter of each egg is ≈ 1.6 mm. (A) Early stage, < 24 h post fertilization; note large sphere of yolk and surrounding perivitelline space. (B) Late stage, > 48 h post fertilization; note curled embryo with small yolk sac.

(a)   **(b)**
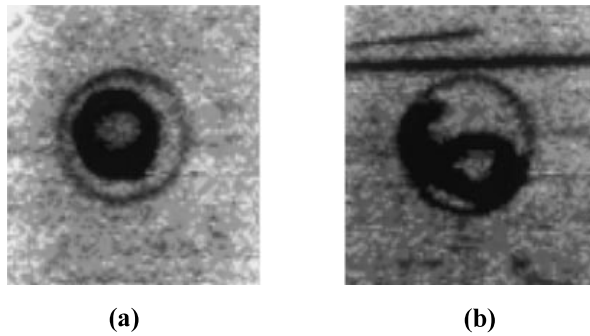
eggs. This, of course, depends on the vertical distribution of eggs and, in turn, the terminal velocity of eggs and water-column mixing (Sundby, 1991; Cambalik, 1993). One may assume, however, that pelagic eggs which, by definition, are positively buoyant, will monotonically increase in concentration towards the surface, as is the case for the Atlantic menhaden (Cambalik, 1993) and northern anchovy (Pommeranz and Moser, 1987). Cambalik (1993) used a one-dimensional, time-dependent model of physical mixing with laboratory-derived estimates of terminal ascent rate of Atlantic menhaden eggs to show they achieve such a distribution within 12 h after spawning when released as deep as 30 m. Hence, we hypothesized that

estimates of volume-based concentration at 3 m from CUFES would be related to area-based estimates from a CalVET for northern anchovy and Pacific sardine. This was the case for both species, with correlation coefficients for ln-transformed abundance of 0.85 and 0.62 for anchovy and sardine, respectively. The slope of the relation for both untransformed and ln-transformed data was greater for sardine than for anchovy. This may be due to the greater size, hence more efficient retention, of the larger sardine egg but also to differences in the vertical distributions of eggs of these two species, for which comparative data are lacking. A recent comparison of CUFES- and CalVET-derived estimates of the concentration (eggs m^{-3}) of pilchard, *Sardinops sagax*, eggs off the west coast of South Africa yielded an r^2 of 0.82 ($n = 153$) for untransformed data (C.D. van der Lingen and D.M. Checkley, Jr, unpublished data). We are now attempting to explain the variance in the relation between volume- and area-based estimates of egg abundance by (a) measuring the vertical distribution of eggs and (b) predicting this distribution from their measured terminal ascent rate (Cambalik, 1993) and a mixing model forced by meteorological and oceanographic variables able to be measured routinely at sea. To this end, a CTD should be deployed with the CalVET net to measure physical structure of the water column. Such measurements were not made during JD9603.

The average concentration of eggs in a large area or population is needed at times, as for the daily egg production method (Lasker, 1985). This is done at present by taking CalVET collections at stations in a predetermined grid. The precision of the estimate of

Table 3. Response of optical plankton counter to individual particles. Age for eggs of Atlantic menhaden, *Brevoortia tyrannus*, is days post fertilization (dpf). Hatching occurred at 3 dpf. *ESD*[a] is from semi-empirical equation of Herman *et al.* (1991).

Particle type	Optical Plankton Counter Response	
	Digital size units (dsu)[b]	*ESD* (mm)
Brevoortia tyrannus		
Live eggs, age 1 dpf	44 ± 2 (15)	≈0.70
Live eggs, age 2 dpf	53 ± 1 (22)	≈0.78
Live eggs, age 3 dpf	81 ± 3 (32)	≈0.97
Live larvae, age 3 dpf	47 ± 3 (38)	≈0.73
Live larvae, age 4 dpf	56 ± 3 (37)	≈0.80
Live larvae, age 5 dpf	60 ± 3 (40)	≈0.82
Stained, live eggs, age 3 dpf	186 ± 2 (80)	≈1.5
Formalin-fixed eggs, age 1 dpf	102 ± 4 (36)	≈1.1
Polystyrene beads (opaque), 1.4–1.6 mm diameter	206 ± 23 (96)	≈1.6

[a] Equivalent spherical diameter.
[b] Data are mean ± SE.

mean egg abundance depends on the number of samples. CUFES may allow a significant increase in precision of this estimate for the following two reasons.

First, much more water potentially with eggs is sampled when CUFES is deployed on and between stations. In JD9603, for example, 91 stations were occupied at ≈4 km spacing along transects. CalVET and CUFES samples were taken simultaneously at each station. Between stations, CUFES samples were acquired during 3–5 min intervals. A CalVET tow from 70 m to the surface filters ≈3.5 m^3 of water; in 5 min, CUFES filters approximately the same volume (≈3.2 m^3) from 3-m depth. The total number of CUFES samples in the 5.6-day cruise was 1396 and the total number of eggs collected was 10 120 for Pacific sardine and 1863 for northern anchovy. At the 91 stations, CalVET collections yielded 518 sardine and 85 anchovy eggs. The ratio of eggs collected by CUFES to those collected by CalVET is 20 and 22 for sardine and anchovy, respectively. The standard error, $SD(\bar{x})$, a measure of precision, depends on n, the number of samples: $SD(\bar{x}) = SD(x)/\sqrt{n}$. Hence, assuming the $SD(\bar{x})$ is similar between CUFES and CalVET and that $SD(\bar{x})$ changes little from CalVET (n = 91) to CUFES (n = 1396), the standard error will decrease by $(n_{CalVET}/n_{CUFES})^{1/2} \approx 4.0$. This was, in fact, the case for anchovy ($SD(\bar{x})$, 0.33–0.078). But the decrease was less for sardine (0.93–0.68) due in large part to its higher sample variance. This, in turn, is due to the greater range of values from CUFES than CalVET, indicating a highly contagious distribution. This decrease, however, does not take into account the increased autocovariance associated with CUFES samples and hence may be an overestimate of the gain in precision (Thompson, 1992), an issue now under investigation (N. Lo, Southwest Fisheries Science Centre, NOAA, La Jolla, California, pers. comm.).

Second, analysis of CUFES samples at sea enables adaptive sampling with consequent increases in the precision of abundance estimates and survey efficiency. There was strong agreement (r^2, 0.88–0.97) between estimates of egg abundance made at sea at the time of sampling and ashore with detailed microscopic analysis of sardine and anchovy eggs from JD9603. Thus, sea counts are reliable indicators of egg abundance. Adaptive sampling will lead to a gain in precision of the estimate of mean abundance if eggs are highly aggregated, as is most often the case, and if they occur within a limited spatial area (Thompson, 1992). In the first consideration, adaptive sampling will cause the survey pattern to include additional samples taken after one meets a sample criterion, a critical number of eggs per unit sample time or volume. In the second,

the survey pattern will be modified to avoid prolonged sampling of waters not likely to contain eggs based on prior CUFES samples and other information, such as the temperature dependence of egg abundance, AV-HRR SST, and 3-m temperature. Adaptive sampling using CUFES may lead to significant gain in precision relative to conventional sampling on a fixed grid, leading to the more efficient use of ship time. In fact, when entering a patch of menhaden eggs in our present investigations, eggs are first detected visually on the video monitor and this information is used to initiate more frequent sample collection.

For use of CUFES in the DEPM, eggs of all developmental stages must be sampled with equal efficiency. This criterion, in turn, depends on the vertical distribution of eggs of different stages of development. This distribution may vary between species and will depend on depth of spawning and dependence of the ascent rate on stage of development and environmental conditions. All developmental stages, save the first, of the Pacific sardine appeared to have been sampled with equal efficiency by CUFES and CalVET nets in JD9603 (Fig. 4). Of the few (n = 34) stage-one eggs collected, nearly all (n = 31) were caught by the CalVET net. This bias may have been due to chance and/or the occurrence of stage-one eggs below the CUFES pump depth. Stage-one eggs are rarely collected by nets in DEPM surveys, presumably due to their highly aggregated distribution, and thus are not used to estimate egg production or mortality (Lo *et al.*, 1996). Comparison of CUFES and areal abundances with regard to stage of development will be necessary whenever CUFES is to be used for stock assessment using the DEPM.

Few data exist for the distribution of eggs of the Atlantic menhaden off North Carolina, the primary spawning region of this species (Judy and Lewis, 1983). Checkley *et al.* (1988) and Warlen (1994), based on the collections of eggs and young larvae, indicate spawning to occur between the west wall of Gulf Stream and the mid-shelf front, a region delimited by sea surface temperatures of ≈21–15°C and salinity 36.2–36.5. CUFES results presented here are consistent with this distribution. In addition, eggs tended to be found in water with ≥ 0.1 *μg* chl *a* l^{-1}. Together, these data are consistent with spawning near the western wall of the Gulf Stream in recently upwelled, nutrient-rich water (Checkley *et al.*, 1988). The data are as yet too few to test the hypothesis that spawning occurs during storms (ibid.), although CUFES facilitates such a test due to its high efficiency (volume sampled / survey time) and ability to operate during storms. Data from 1994 to 1995 (not presented here)

show patches of menhaden eggs to have occurred in the same geographical region as in 1993–94 (Fig. 6) and hence spawning may, in addition, be related to bathymetry. This may be evolutionarily advantageous owing to the placement of eggs in a region with predictably favourable transport and development (Checkley *et al.*, 1988; F. Werner, University of N. Carolina, Chapel Hill, pers. comm.). Sampling and modelling studies are under way to address this idea.

At present, data on egg distributions from CUFES are obtained from the microscopic analysis of samples. We believe, however, that automation of the counting of target eggs in CUFES is feasible. Analysis of our OPC data by differencing size-frequency distributions within and near patches of eggs clearly showed a peak attributable to menhaden eggs (Fig. 8). Integration beneath the peak yielded an estimate of menhaden eggs surprisingly similar to the actual number of eggs known to have passed through the OPC and retained by the MSC during these sampling periods. Finally, the mean and range for digital size of eggs in nature (Fig. 8) corresponded well to the digital size of eggs spawned and reared in the laboratory (Table 3), particularly for early to mid-stage eggs, which dominated these patches. The increase in digital size of eggs and larvae during development is most probably a function of accumulated tissue, including melanophores, and orientation of the embryo and larva. Similar results have been reported for the Baltic cod (Wieland and Köester, 1996). Our comparison between the OPC and microscopic counts was made using samples of known abundance of menhaden eggs, based on prior, microscopic analysis. We are developing real-time, video-based egg identification and counting, either with or without use of the OPC. Images of early and late-stage eggs of the Atlantic menhaden (Fig. 9) are amenable to computer analysis and identification. While microscopic examination of net samples will always be needed in conjunction with data from electronic devices, the use of the latter appears feasible in the next several years. This would further enhance the efficiency of CUFES and its use in the DEPM and basic science studies.

CUFES has not only applied merit but is useful for basic studies. The processes responsible for survival and, conversely, mortality of the pelagic eggs of fish operate on scales from less than metres to greater than tens of kilometres. For example, eggs that accumulate in windrows of Langmuir circulations may be more susceptible to predators (Matsushita, 1991). On a larger scale, eggs spawned in water upwelled at the Gulf Stream Front during storms may exhibit optimal development and survival (Checkley *et al.*, 1988).

CUFES affords the possibility of investigating the distribution of eggs over this range of spatial scales simultaneously.

ACKNOWLEDGEMENTS

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