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ANALYSIS OF THE VERTICAL DISTRIBUTION OF THE ABUNDANCE OF SMALL PELAGIC FISH LARVAE IN THE GULF OF CALIFORNIA USING SUBMARINE VIDEOCAMERAS

Análisis de la distribución vertical de la abundancia de larvas de peces pelágicos menores en el Golfo de California mediante videocámaras submarinas

RESUMEN. Se utilizaron dos tipos de videocámaras submarinas para estudiar la distribución y abundancia vertical de larvas de los peces pelágicos menores *Engraulis mordax*, *Etrumeus teres* y *Sardinops sagax* a 1 m de resolución, en una localidad en el norte del Golfo de California con condiciones de calma y alta densidad de sardinas adultas. La mayor abundancia promedio (900 larvas m⁻¹ min⁻¹) se encontró inmediatamente arriba de la termoclina (33 m) y la picnoclina (36 m), aparentemente no asociada al máximo de clorofila detectado en superficie, ni a la mayor densidad de peces adultos (10 -20 m). Las observaciones con video permitieron determinar la distribución vertical a una resolución imposible de obtener mediante muestreos con redes; sin embargo, esta es una técnica poco útil en zonas con elevada velocidad de las corrientes.

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Marine pelagic fish larvae inhabit a three-dimensional space where there are pronounced vertical and horizontal gradients in temperature, light, food supply, and currents. These vertical environmental gradients can affect the vertical distribution, abundance, survival, and aggregation behavior of ichthyoplankton (Leis, 2004). Using current technological

capabilities, the environmental gradients can be measured in small spatial scales (centimeters to meters) but the acquisition of biological information that would match such small spatial scales is highly challenging to obtain. Most ichthyoplankton studies depend on nets that provide integrated samples (at specific strata) to infer broad fish egg and larvae vertical distribution patterns (Wiebe & Benfield, 2003). Light traps are occasionally used but such studies take two or at most three levels per sampling location with considerable different species composition than zooplankton collected with nets (Brogan, 1994a, b). Modern "high-tech" nets that open and close the nets electronically like the MOCNESS or BIONESS systems provide multiple stratified levels per trawl (up to 20 nets but usually nine are used) (Wiebe & Benfield, 2003). However, those systems are expensive, time consuming, and provide little evidence of *in situ* behavior of the early life history of demersal, benthic, and pelagic fishes due to their relatively coarse vertical sampling resolution (several meters width strata). Thus, the acquisition of *in situ* high resolution information on vertical distribution and abundance of fish larvae has been for long time recognized as a critical methodological problem in zooplankton ecology (Wiebe & Benfield, 2003).

Hydroacoustic surveys can provide detailed records of zooplankton density in vertical space scales of centimeters (> 20 cm) (Gómez-Gutiérrez & Robinson, 2006). However, it is virtually impossible to identify the species based exclusively on the echo-information without complementary information from nets and/or video cameras to detect and identify the numerically dominant organisms from the sound scattering layers (Wiebe & Benfield, 2003). Unfortunately, fish larvae usually have low absolute densities in the zooplankton community. The lack of rigid structures or a not yet developed swim bladder to be detected by echosounder sound, make the use of hydroacoustic techniques particularly unsuitable to estimate distribution and abundance of fish larvae. Greene and Wiebe (1990) and Ben-

field *et al.* (1996) demonstrated the utility of Remote Operated Vehicle (ROV) video observations to study micro distribution of zooplankton and micronekton that numerically dominate the zooplankton community structure.

In November 2005 an oceanographic cruise was carried out to estimate distribution and abundance of small pelagic fish (19 oceanographic stations) measuring the oceanographic conditions and biological samples from plankton and micronekton (Aceves-Medina *et al.*, 2009). Here we show that under exceptional calm observational conditions (current speeds $<50 \text{ cm s}^{-1}$), submarine video camera observations can be useful to describe high resolution fish larvae vertical distribution ($<1 \text{ m}$) estimating their abundance and observing their *in situ* behavior, previously observed only under laboratory conditions (Hunter, 1981). In future studies, such *in situ* ROV video camera observations may help to understand the fish larvae habitat preferences during their transient meroplanktonic phase.

Two high resolution submarine video cameras were deployed to observe zooplankton and micronekton at 46 locations where an hydro-acoustic survey (SIMRAD EY60, 120 kHz, split beam) showed dense sound scattering layers at the north and central part of the Gulf of California (November 2005) (Fig. 1). The video-camera system used included: 1) a ROV, Seabotix, equipped with color and black & white video cameras, underwater lamp, and temperature and depth sensors and 2) a Multi SeaCam camera (Deep Sea Power & Light, lens $f = 2.8 \text{ mm}$, field depth 0.1 m to infinite) equipped with a submarine lamp Ikelite of 50 Watts attached either to the ring of a 5-m length conical zooplankton net (with black mesh nets 333 μm , 0.25 diameter and 0.75 cm length cod-end) or to a metallic base with a 20 kg weight.

At an oceanographic station (E41), located at 30.05°N , 112.54°W and carried out between November 25 (22:00 h) and November 26, (02:15 h), 2005 (Fig. 1), we detected a dense sound scattering layer using a scientific echosounder that simultaneous ROV video-camera observations identified as zooplankton aggregations and dense schools of

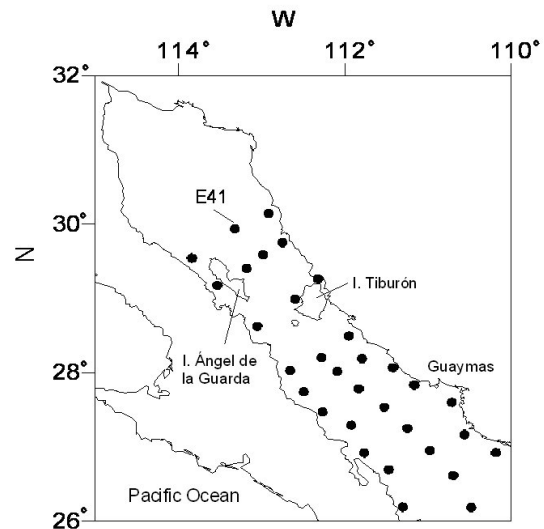


Figure 1. Sampling stations during the oceanographic cruise (November 18 to December 2, 2005) showing the location of the sampling station E41.

adult Pacific sardines (*Sardinops sagax*) (Fig. 2a). Zooplankton samples collected with a 1-m ring diameter drifting net (DN, 10 min duration) equipped with the Multi SeaCam videocamera showed the presence of larvae and adults of small pelagic fish. The net was sent to the depths of high plankton densities (detected as a dense sound scattering layer with the echosounder), sampling from the surface to 40 m depth water column as homogeneously as possible while the ship was drifting. During this period the video camera attached to the net showed large numbers of static and actively swimming white and opaque slender fish larvae (Fig. 2b). The zooplankton sample obtained was analyzed immediately onboard. The fish larvae collected and observed on the video were identified as members of the family Clupeidae and Engraulidae. Later we sent the ROV to a maximum depth of 50 m to variable downward and upward speed of about 2 m min^{-1} (Fig. 2b). All were seen at real time using a 91 cm flat Sony color television and recorded on a DVD for further counting of fish larvae. Exceptionally calm *in situ* conditions allowed notably clear zooplankton observations in the station E 41, but we did not measure the *in situ* current speed. Based on our previous experience of three oceanographic cruises at Bahía Magdalena, where Acoustic Doppler Current Profiler, hydroacoustic and video-cameras

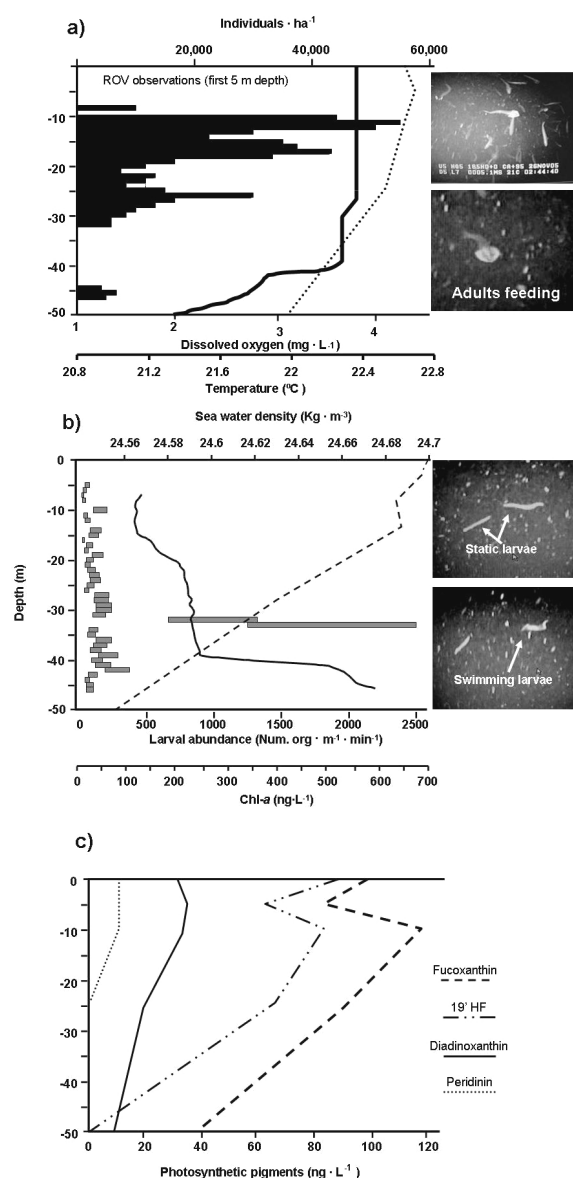


Figure 2. Measured variables at oceanographic station E41: a) Acoustic vertical profiles of abundance of small pelagic fish adult as detected by acoustics (black bars), dissolved O₂ profiles (dotted line), temperature (continuous line), and ROV images of adult pacific sardines (*Sardinops sagax*) feeding near surface. Video complement gaps on acoustic information in surface; b) Vertical profile of fish larvae time-dependent abundance using the ROV (grey bars, inds. min⁻¹), vertical profile of Chl-a (dashed line), sea water density (solid line) and, ROV images of sardine and/or anchovy larvae; c) Vertical distribution of accessory photosynthetic pigments.

were used simultaneously, we observed zooplankton clearly at current speed < 50 cm s⁻¹ (Gómez-Gutiérrez & Robinson, 2006; Robinson *et al.*, 2007). Thus, in the E41 station it is likely that current speed was < 50 cm s⁻¹.

Using the ROV videotape, two independent observers simultaneously counted the number of larvae detected per 1-m layer bin guided by the depth displayed on the screen of the video while a third person controlled the video recorder at slow motion. Because the video-camera moved at different speeds at each depth layer, larval abundance was standardized as average number of fish larvae per meter width layer per minute (inds. m⁻¹ min⁻¹) dividing the number of larvae observed at each 1 m stratum in the time spent by the camera at such 1 m stratum. The average and standard deviation of fish larvae counted by each observer was not significant different (t-test, $p < 0.05$; Observer 1 = 5.6 larvae m⁻¹ min⁻¹ with a standard deviation of 2.44 larvae m⁻¹ min⁻¹ and observer 2 = 6.7 larvae m⁻¹ min⁻¹ with a standard deviation of 2.41 larvae m⁻¹ min⁻¹). Additionally, we did a standard oblique Bongo trawl (333 and 505 μ m mesh net) and a 10 minutes surface horizontal trawl (SN) with a conic 0.6 m diameter net (505 μ m mesh net). These plankton nets had digital flow meters to estimate the filtered water volume. All the fish larvae were sorted out from the complete Bongo 505 μ m and surface nets samples and standardized as inds. m⁻³. For the fish larvae collected with the drifting net (non quantitative sample) the abundance was reported as total number of larvae collected in the tow and expressed in relative abundance (%) (Table 1). At each oceanographic station, including the E41 station, we did a CTD cast (General Oceanics Mark III) to 200 m depth and sampled seawater with 5 L Niskin bottles at 0, 5, 10, 25 and 50 m depth to measure dissolved oxygen concentration with an oxymeter YSI-1556. From each Niskin bottle we filtered 350 ml of water with GF/F filters (0.7 μ m) and froze them with liquid nitrogen to estimate photosynthetic and accessory pigment concentration using High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FD) (Vidussi *et al.*, 1996). All these observations were similarly done in the rest of the 18 oceanographic stations, but only at E41 station the low current speed conditions and the large density of fish larvae (of a relatively large size) allowed to do detailed observations of behavior and visual estimations of small pelagic fish larvae densities.

Table 1. Fish larvae species abundance collected at station E41. (BN) = Bongo 505- μ m net; (SN) = surface neuston net with standardized abundance to inds. m^{-3} . (DN) = Total number of larval collected with drifting net expressed in relative abundance (%).

Family	Fish larvae species	Abundance per net type					
		Ind m^{-3} or total number			Relative abundance		
		BN	SN	DN	BN	SN	DN
Mictophidae	<i>Benthoosema panamense</i>	472.1	284.3	119	52.3	69.0	48.4
Engraulidae	<i>Engraulis mordax</i>	244.0	52.2	96	27.1	12.7	39.0
Scombridae	<i>Scomber japonicus</i>	79.6	11.6	7	8.8	2.8	2.9
Clupeidae	<i>Etrumeus teres</i>	5.3	29.0	6	0.6	7.0	2.4
Clupeidae	<i>Sardinops sagax</i>	37.1	11.6	5	4.1	2.8	2.0
Gobiidae	<i>Lythrypnus</i> spp.	15.9	11.6	4	1.78	2.8	1.6
Paralichthyidae	<i>Citharichthys xanthostigma</i>	10.6	5.8	6	1.2	1.4	2.4
Serranidae	<i>Pronotogrammus multifasciatus</i>		5.8			1.4	
Mictophidae	<i>Triphoturus mexicanus</i>	10.6			1.2		
Triglidae	Triglidae	5.3		1	0.6		0.4
Synodontidae	<i>Synodus</i> sp.	5.3		1	0.6		0.4
Fistularidae	<i>Fistularia corneta</i>	5.3		1	0.6		0.4
Nomeidae	<i>Cubiceps paucirradiatus</i>	5.3			0.6		
Albulidae	<i>Albula</i> sp.	5.3			0.6		

Video records indicated that most small pelagic fish larvae were static with their thin body straight (suggesting an energy saving strategy behavior under calm current speed conditions), but in response to mechanical and/or light stimulation from the videocamera they invariably escaped adopting a typical S-shape with short undulating swimming movements, followed by a sudden stretching movement that displaced the larvae forward (Fig. 2b). Similar behavior was observed in sardine larvae under laboratory conditions (Hunter, 1981). The fish larvae collected simultaneously with the drifting net (equipped with the video-camera) at the core of high larvae density had a standard length range of 17-21 mm. Fish larvae of this size range swam independently of each other with considerable distance between them with no evidence of schooling behavior. This suggests schooling behavior may develop in larger fish larvae. Ichthyoplankton taxonomic composition from the three zooplankton nets included 14 species from 12 families (Table 1). The myctophid

Benthoosema panamense (48%-69%), northern anchovy *Engraulis mordax* (12%-39%), jack mackerel *Scomber japonicus* (2.8%- 8.8%), round herring *Etrumeus teres* (0.6%-7.0%), and Pacific Sardine *Sardinops sagax* (2.03%-4.12%) accounted for 93%-95% of the total fish abundance for each type of net (Table 1). We did not collect eggs with any net used that otherwise would suggest recent spawning of the adult sardines observed at E41 oceanographic station or nearby locations.

From all fish larvae species collected, only *E. mordax*, *E. teres*, and *S. sagax* matched the size and body shape observed in the videos. From the zooplankton samples the relative abundance of small pelagic fish larvae were *E. mordax* (77 %), *E. teres* (13%, particularly abundant in the neustonic sample), and *S. sagax* (10%) (Table 1). ROV videos (starting at 22:00 h local time) showed dense adult Pacific sardine schools feeding near the surface with the highest densities in the strata between 5 - 20 m depth and lower densities at

deeper strata (Fig. 2a). Because the transducer of the echosounder was located 4-m below the sea surface and accounting for near field effect, acoustic data are available only for layers > 6 m depth. Using criteria of scattering volume < -50 dB and 50 pings of echogram analysis to detect juvenile and adult small pelagic fish schools (Robinson *et al.*, 2007), the hydroacoustic information recorded at the E41 station confirmed the video-camera observations that most of the adult fish abundance (inds. ha^{-1}) was located between 10 and 20 m depth (Fig. 2a).

Fish larvae were not detected with the ROV video-camera in the first 4 m depth. Larvae were detected in low time-dependent densities (46 fish larvae $\text{m}^{-1} \text{min}^{-1}$) between 5 - 26 m (Fig. 2b). The average of fish larvae time-dependent abundance increased to 103 fish larvae $\text{m}^{-1} \text{min}^{-1}$ between 27 - 31 m depth showing the highest average densities (650 - 900 fish larvae $\text{m}^{-1} \text{min}^{-1}$) between 32 - 33 m depth. The density of fish larvae decreased progressively at deeper strata (Fig. 2b). From the video-camera information it is not possible to estimate volume or area sampled. Thus, fish larvae abundance as a function of time is not comparable to densities estimated with traditionally net methods (inds. m^{-3} or inds. m^{-2}). The video count method can estimate unusually high maximum extrapolated larvae fish densities (compared with net collection) when the video camera spent little time at each 1-m depth bin and abundance was extrapolated to one minute (*i.e.* 1200 larvae $\text{m}^{-1} \text{min}^{-1}$). This methodological problem can be solved if the video camera is deployed slowly ($< 1 \text{ m min}^{-1}$) to avoid extrapolation of larval density to 1 minute intervals of observations per 1 m stratum.

The apparently inverse vertical distribution pattern of adults and larvae, corroborated with video observations (Fig. 2b), might suggest high larval mortality from predation by the schools of adult sardines located near surface waters or spatial ontogenetic segregation to avoid cannibalism. Near surface feeding of *S. sagax* and *E. mordax* during night time is a common observation at several sea regions (Krutzikowsky & Emmett, 2005; Robinson *et al.*, 2007). Although several studies have not been detected large fish larvae (like those ob-

served in the video camera) in the stomach of adult small pelagic fish, Hayasi (1967) detected intensive cannibalism on eggs and small larvae of anchovies and sardines, and Buttler (1991) showed robust statistical evidence of cannibalism on anchovy and sardine larvae. Because small pelagic fish are not selective filtering feeders, it has been largely discussed if the northern anchovy can filter their own eggs and early larvae, thus they also may feed on larger fish larvae (Buttler, 1991). Hunter & Kimbrell (1980) reported that because of the thin integument of fish larvae and the rapid adult digestion rates, fish larvae are rarely found in the stomach content of adult anchovies. Our most likely explanation is that the vertical distribution pattern of small pelagic fish larvae in E41 responds to a complex vertical and horizontal ontogenetic segregation among fish eggs, larvae, and adults, as a potential strategy to avoid cannibalism.

The highest small pelagic fish larvae abundance was detected just above the thermocline and pycnocline (Fig. 2 a, b), but not associated with dissolved oxygen concentration (4 - 4.4 mg L^{-1} in the first 25 m depth, equivalent to 75% oxygen saturation, and decreased to 3 mg L^{-1} at deeper strata) nor the depth of the chl-*a* maximum concentration detected at surface (Fig. 2c). In the southwestern part of the Gulf of California (Bahía de La Paz) the higher concentrations of fish larvae, including *Opisthonema* spp., was located above the pycnocline, which is the strata with maximum stability (16 - 48 m) (Sánchez-Velasco *et al.*, 2007). In the coast of California the higher abundance of *S. sagax* was detected between 22 and 45 m depth (Watson, 1992). The average depth of maximum chl-*a* concentration in the first 75 m depth in the 26 oceanographic stations was 4 m (standard deviation = 5.6 m and about 50% of the stations had a maximum chl-*a* concentration at surface). This is a relatively shallow depth considering that November is a transition period where the mixing layer begins to develop. In July 2007 the maximum of chl-*a* was 19 m depth and in January 2007 30 m depth (Gómez-Gutiérrez *et al.*, in press) suggesting that during November irradiance likely caused a smaller photo-inhibition process than in July. The fucoxanthine and 19-hexanoy-

loxyfucoxanthin (19-Hf) phytoplankton pigments (indicators of diatoms and cyanobacteria–prochlorophyta, respectively) (Jeffrey, 1974; Goericke & Repeta, 1993) had a pattern similar to chl-*a* suggesting that those groups were the most abundant phytoplankton components at this location (Fig. 2c). We did not detect significant association between the maximum densities of fish larvae and phytoplankton, which in theory should provide a suitable environment for feeding larvae (Fig. 2b). To our knowledge, except Sánchez-Velasco *et al.* (2007), there is no other study of vertical distribution of fish larvae in the Gulf of California to compare with our observations.

Submarine videos allowed us to observe *in situ* small pelagic fish larvae in static resting behavior and vertical distribution at an unprecedented resolution (1 m depth). However, video-camera observations may overestimate fish larval densities compared with conventional net method estimations and was practically useless in the other 45 locations where we used the ROV during the oceanographic cruise (Fig. 1) because intense current speed conditions prevailed that prevented us to do reliable identification of zooplankton. High current speeds ($>50 \text{ cm}^{-1} \text{ s}^{-1}$) may restrict video-camera observations to enclosed regions or video recording during transient calm sea conditions that can be specifically selected from tide tables to increase the probability of obtaining adequate observational conditions and increase the number of behavioral observations of fish larvae *in situ*. Even with these technical limitations, it is clear that video records are valuable complement of standard net sampling methods in the study of *in situ* fish larvae behavior that can not be obtained by other means in the field.

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