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ORIGINAL ARTICLE

## Ichthyoplankton dynamics in a highly urbanized estuary

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### Abstract

Spatio-temporal distribution and composition of ichthyoplankton assemblages were studied in the Golden Horn estuary (Istanbul) over a 10-month period. Environmental parameters were considered to determine the environmental status in different parts (upper, middle and lower) of the estuary. The ichthyoplankton composition of the Golden Horn estuary consisted of 23 species and was dominated by *Mullus* sp., *Diplodus* spp. and *Liza* sp. The largest densities of fish eggs and larvae were found in September 2009 with 786.4 ind. 100 m<sup>-3</sup> and 355.9 ind. 100 m<sup>-3</sup>, respectively. As supported by the multivariate analysis, most species showed a seasonal pattern, with the presence of higher densities during summer and winter. Moreover, the spatial pattern showed that ichthyoplankton distribution and diversity was relatively high in the lower part of the Golden Horn and gradually decreased through the upper parts. Canonical correspondence analysis revealed that spatial changes in depth and water clarity were the main factors forcing larval assemblage distribution and leading to a decrease in density and diversity of fish larvae through the upper part of the estuary. For the seasonal changes, sea surface salinity and chlorophyll *a* were the main factors in shaping the structure of the larval assemblage and increasing sea surface temperature lead to an increase in the density and diversity of fish larvae.

**Key words:** *Early life stages, environmental factors, Golden Horn estuary, larval assemblage, nursery, urbanization*

### Introduction

Estuaries are transition zones between the sea and rivers with an often sharp salinity gradient (McLusky & Elliott 2004). These naturally sheltered zones possess characteristics favourable to many fishes, such as shallow water depth, rich food availability and a diversity of habitats (Beck et al. 2001; Whitfield & Elliott 2002). Fish species may inhabit estuaries for their whole life (estuarine species), for a particular period of their life such as marine juveniles (marine stragglers) or only use them as a migration zone (marine migrants – anadromous and catadromous fishes). As nursery areas, the importance of estuaries for the early life stages of many fish species has often been emphasized (Elliott et al. 2007; Courrat et al. 2009). The dynamics of larval fish assemblages may be a good indicator of the behavioural ecology of fish populations. The developmental stage of fish particularly responds to several physical and biological variables

(namely hydrological conditions, seasonal variability, and spawning preferences of adults) with direct consequences for the survival rates and healthiness of future fish stocks (Lasker 1981; Miller 2002).

Estuaries also provide various ecosystem services: they function as waterways and fishing zones, and they are a source of freshwater supply. All these benefits ultimately intensify urban development around estuaries. However, a number of anthropogenic factors have resulted in habitat loss, compromised ecosystem function and changed species composition in estuaries (Borja & Dauer 2008). These factors include changes in land use, elevated nutrient loads, increasing inputs of metals and organic contaminants, and the resultant high microbial activity (McLusky & Elliott 2004; Jones 2006). Recent freshwater diversions, for example those caused by dam-building, have increased the residence time of the water mass in estuaries and amplified the adverse effects of all these anthropogenic disturbances (Bianchi 2007).

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The Golden Horn estuary, Istanbul's waterfront area, has had significant socio-economic importance for centuries based on the rich natural resources of the area. In particular, the estuary and its tributaries have been noted for their rich fish biodiversity that have even included top predators such as dolphins and bluefish. From ancient times until the 1950s the estuary was an important fishing ground (Güvengiriş 1977; Tekin 1996). The estuary remains a valuable natural asset despite the modern city of Istanbul expanding on both sides. However, as is the case in most urban estuaries (McLusky & Elliott 2004), the Golden Horn has suffered dramatic ecosystem shifts since the early 2000s as a result of population increase and inadequate infrastructure and the subsequent eutrophication and anoxia caused by increasing domestic and industrial wastewater effluent (Aslan-Yılmaz et al. 2004). This study focused on the distributional patterns of fish eggs and larvae in the Golden Horn estuary. In this context, two main objectives were specified: (1) determination of the ichthyoplankton density and distribution, and (2) analysis of the dynamics of the ichthyoplankton in relation to environmental variables. For this purpose, seasonal and regional changes of the ichthyoplankton assemblage were observed. Environmental parameters were considered to determine environmental status in different parts (upper, middle and lower) of the Golden Horn estuary. Differences in the environmental status and influence on the ichthyoplankton assemblage were discussed.

## Materials and methods

### Study area

The Golden Horn is, as its name suggests, a horn-shaped estuary (41°01'N and 28°41'E) located in a southwest–northeast direction between the Bosphorus (Istanbul Strait) and two tributaries (Alibey and Kağıthane; Figure 1). It is 7.5 km long with a surface area of 2.6 km<sup>2</sup> and is widest at the entrance (1010 m). The estuary narrows towards the upper part: its width is 700 m in the mid part and 200 m in the upper part around the streams. The maximum depth is 40 m in the lower estuary near the Galata Bridges, but decreases sharply after the Atatürk Bridge (35 m) through the shipyard (15 m) and continues to decrease towards the upper estuary (4 m) (Figure 1). There is a vertically sharp salinity gradient due to the brackish waters originating from the Black Sea (~18–20), saline waters from the Mediterranean Sea (~38) and freshwater from the Kağıthane and Alibey tributaries (Müftüoğlu 2008). The current annual freshwater input from the tributaries ( $3 \times 10^5 \text{ m}^3 \text{ year}^{-1}$ ) is quite low, equal to the daily stream input ( $3 \times 10^5 \text{ m}^3 \text{ day}^{-1}$ ) of the period

before dam construction (Kor 1963; Müftüoğlu 2008). The surface discharges into the Golden Horn were abolished and have been connected to a deep discharge facility in Istanbul Strait via a connector system since 2002 (Aslan-Yılmaz et al. 2004).

### Sample collection and analysis

Sampling was carried out monthly from April 2009 to January 2010 at four stations. Station 1 (S1) was located in the lower estuary exit near the Galata Bridge, where the depth is 40 m, and station 2 (S2) was closer to the Atatürk Bridge, where the depth is 35 m. These two stations were also in an area of intense marine traffic. Station 3 (S3) was in the middle part of the estuary (15 m depth) and influenced by shipyard facilities. Station 4 (S4) was the shallowest sampling point (4 m), located in the upper estuary (Figure 1).

The following environmental variables were measured: sea surface temperature (SST, °C), sea surface salinity (SSS, no unit), dissolved oxygen (DO, mg l<sup>-1</sup>), chlorophyll *a* (chl-*a*, µg l<sup>-1</sup>), dissolved inorganic nutrients (µM), total suspended solids (TSS, mg l<sup>-1</sup>), fecal coliform bacteria (FC, CFU 100 ml<sup>-1</sup>), Secchi disc depth (m). SST and SSS profiles were measured by a SBE 9-11 CTD system. Water samples were collected with 5 l Niskin bottles. DO was measured using Winkler titration onsite upon collection of the samples (APHA 2005). All samples for FC were pre-processed aseptically onboard within 6 h of collection. Membrane filtration was carried out for fecal indicator bacteria detection (APHA 2005). Subsamples with appropriate dilutions were filtered on 0.45 µm cellulose nitrate membrane filters (Sartorius, ref. 13906-50-AJN) using a vacuum filtering set. Filters were then incubated for 24 h at  $44.5 \pm 0.1^\circ\text{C}$  on m-FC medium (Sartorius, ref. 14068-50-N). All blue colonies were accepted as FC. Nutrient samples were pre-filtered on 5 µm syringe filters (Sartorius 17594) and collected in high-density polyethylene (HDPE) bottles that were pre-washed with 5% HCl acid and rinsed with deionized water. TSS samples were filtered through GF/F glass fibre filters (APHA 2005). All nutrient, chl-*a* and TSS samples were kept at  $-20^\circ\text{C}$  until further analysis at the land-based laboratory. On land, chl-*a* was analyzed by the acetone extraction method (Parsons et al. 1984) and measured with a spectrophotometer. Nutrient analyses (nitrate + nitrite, NO<sub>2</sub>+NO<sub>3</sub>-N; ortho-phosphate, PO<sub>4</sub>-P; and silicate, SiO<sub>2</sub>-Si) were calculated using a Bran-Luebbe AA3 auto analyser according to the manufacturer's instructions. Light penetration was measured by a Secchi disc. Precipitation (mm) and maximum and average wind speed (km h<sup>-1</sup>) data were obtained from Boğaziçi

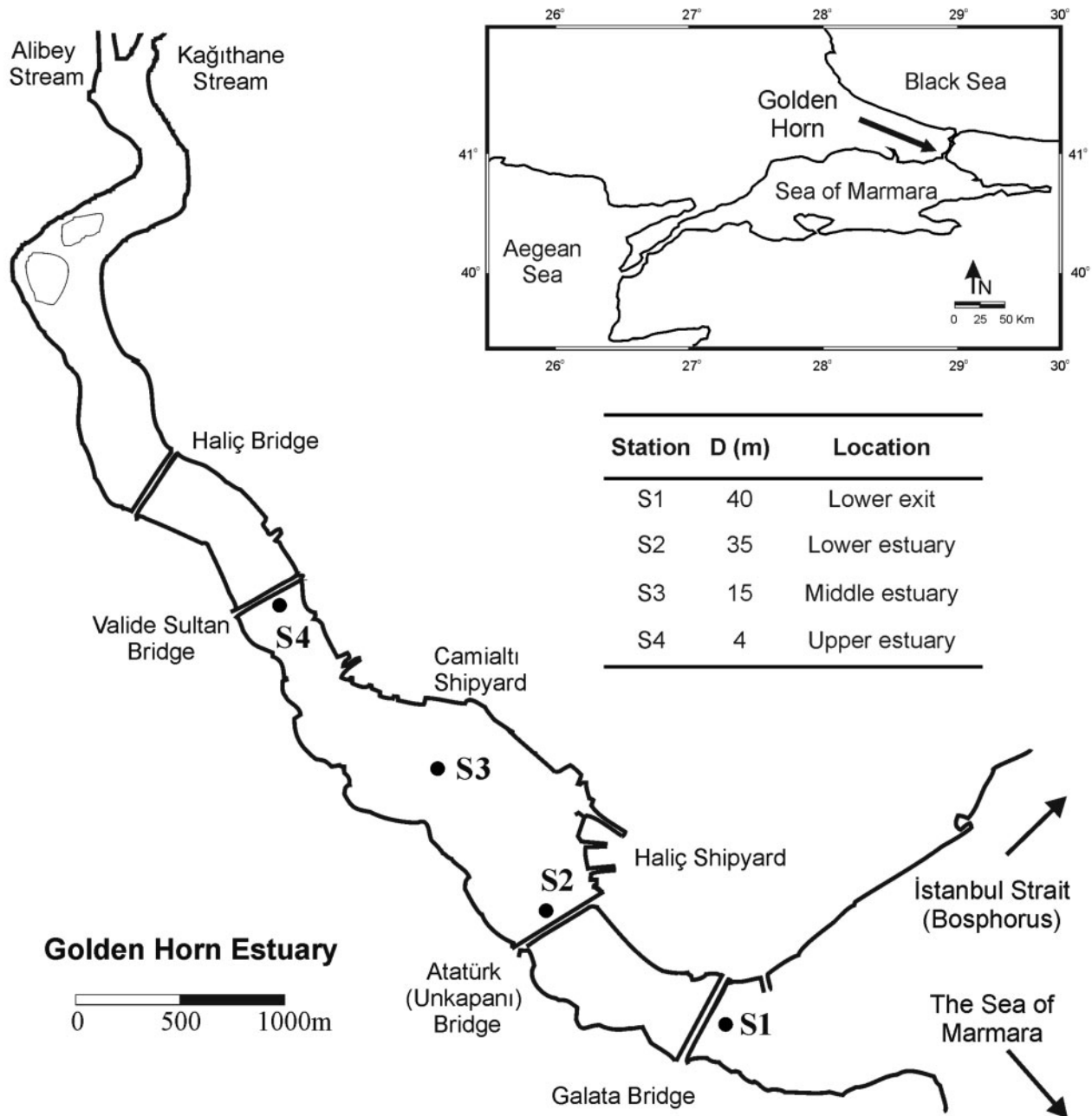


Figure 1. Map of the stations where ichthyoplankton samples were collected between April 2009 and January 2010 in the Golden Horn estuary. The smaller map shows the location of the estuary between the Bosphorus and the Sea of Marmara. The table lists the depth (D) and representative situation of the stations in the estuary.

University, Kandilli Observatory and the Earthquake Research Institute (KOERI).

Ichthyoplankton samples were collected by horizontal tows using a 0.57 m diameter, 500  $\mu\text{m}$  mesh size Nansen net. Horizontal hauling was done in the surface zone (0.5–1 m) for 7 min at a towing speed of 2–4 knots. Samples were fixed in 4% buffered formaldehyde upon collection. In the laboratory after sorting, fish eggs and larvae were identified to the lowest possible taxonomic level. The identification of

fish eggs and larvae was carried out according to D'Ancona (1956), Arim (1957), Demir (1959, 1969, 1974), Dekhnik (1973) and Russell (1976). Density data were standardized as the number of individuals per 100  $\text{m}^3$  (ind. 100  $\text{m}^{-3}$ ).

#### Data analyses

The collected data allowed for the estimation of ichthyoplankton density ( $N$ ), number of species ( $S$ ) and the Shannon diversity index ( $H'$ ). The Shannon

diversity index was calculated with the formula  $H' = -\sum_{i=1}^S p_i \ln p_i$ , where  $S$  is the number of identified fish taxa and  $p_i$  is the proportion of individuals in taxa  $i$ . Non-parametric multivariate techniques were utilized to determine the spatial and temporal patterns of ichthyoplankton distribution and density. Fish eggs and larvae were analysed separately and species with a total density greater than 3% were used to reduce the effects of rare species on the analysis. Density data were transformed to their square root to construct a similarity matrix using the Bray–Curtis coefficient of similarity. Non-parametric multidimensional scaling (nMDS) was used to display a two-dimensional ordination plot with the differences in assemblages (Clarke & Warwick 2001). Data were evaluated according to two established factors: ‘season’ and ‘stations’. The season factor was defined as spring (April and May 2009), summer (June, July and August 2009), autumn (September, October and November 2009) and winter (December and January 2010). Clarke & Warwick (2001) emphasized that a stress value below 0.1 corresponds to a good ordination without any real possibility of misleading interpretation. Analysis of similarities (ANOSIM) was used to identify significance of differences in assemblage groups displayed by nMDS. Univariate and multivariate analyses (Shannon diversity, nMDS and ANOSIM) were applied using PRIMER v6.1.10.

Spatio-temporal changes in hydrography (depth, SST, Secchi disc depth), environmental variables (chl-*a*, DO, nutrients, FC, TSS) and ichthyoplankton variables (density of fish eggs and number of species) were compared across ‘season’ and ‘stations’ by one-way ANOVA. Prior to the analysis of variance, all data were normalized by logarithmic transformation and the normality of data was tested by the Kolmogorov–Smirnov test. The variables that did not cover normal distribution (SSS, larval density and  $H'$ ) were tested by non-parametric Kruskal–Wallis ANOVA analysis. Significant effects shown by ANOVA were further analysed *post hoc* by the Tukey test. Non-parametric Spearman rank correlation was used for the relationship between biological components (eggs and larvae density,  $S$ , and  $H'$ ) and environmental parameters.

The relationship between fish larvae assemblages (dependent variables) and the environmental parameters (independent variables) was analysed by canonical correspondence analysis (CCA; ter Braak 1986). Samples with no fish eggs or larvae were included in the analyses under the category ‘no fish’ to prevent eliminating samples. The minimum possible weight (0.01) was chosen as a density value for the category ‘no fish’. Prior to the analysis, density data were square-root transformed to reduce the weight of the most abundant species and logarithmic

transformation ( $\log x+1$ ) was applied to environmental variables. A forward selection procedure was used to select environmental variables. The significance ( $< 0.05$ ) of the species–environment correlation was tested by a Monte Carlo permutation test (with 999 randomizations). Finally, CCA produces a bi-plot that represents results with environmental variables as vectors, sampling stations as points and species assemblages with their code in the ordination space.

## Results

### Environmental variables

Environmental variables mainly showed significant seasonal variability without any discernible regional pattern except for chl-*a* and Secchi disc depth measurements (Table I). SST was highest in August 2009 and lowest in January 2010 (Figure 2a). The middle estuary was warmer in spring and early summer than the lower estuary. SSS did not show any significant regional or seasonal changes (Table I), was around 18 and mostly uniform at the regional scale. However, in December and January 2010, a sharp decrease in SSS was observed in the upper estuary (Figure 2b) due to heavy precipitation (Spearman rank correlation  $R = -0.636$ ,  $P < 0.01$ ; Figure 2l). Chl-*a*, an indicator of phytoplankton biomass, was the most variable parameter on both seasonal and regional scales (Table I; Figure 2c). Peak values and high fluctuations were observed in spring and summer seasons in the middle estuary.

Table I. One-way ANOVA results of seasonal and regional patterns in environmental and biological data. Abbreviations: see text.

Parameters	Factors			
	Stations		Seasons	
	$F_{3,40}$	$P$	$F_{3,40}$	$P$
Depth	458.45	> 0.01	–	–
SST	0.129	0.942	44.830	> 0.01
SSS*	1.906	0.592	11.699	0.085
Chl- <i>a</i>	14.140	> 0.01	7.349	> 0.01
DO	0.155	0.984	9.208	> 0.01
$\text{NO}_x$	0.102	0.958	80.464	> 0.01
$\text{PO}_4$	1.295	0.290	40.973	> 0.01
$\text{SiO}_2$	0.333	0.801	51.402	> 0.01
FC	0.626	0.602	9.609	> 0.01
Secchi disc	25.181	> 0.01	0.878	0.461
TSS	2.600	0.066	9.290	> 0.01
$S$	14.654	> 0.01	1.496	0.231
$H'$ *	20.091	> 0.01	4.226	0.238
Fish egg density	34.571	> 0.01	1.393	0.260
Fish larvae density*	14.195	> 0.01	4.261	0.234

\*Non-parametric Kruskal–Wallis test results for SSS,  $H'$ , and fish larvae density, which showed non-normal distribution. d.f. = 2 for fish larvae density due to the absence of samples from S4.



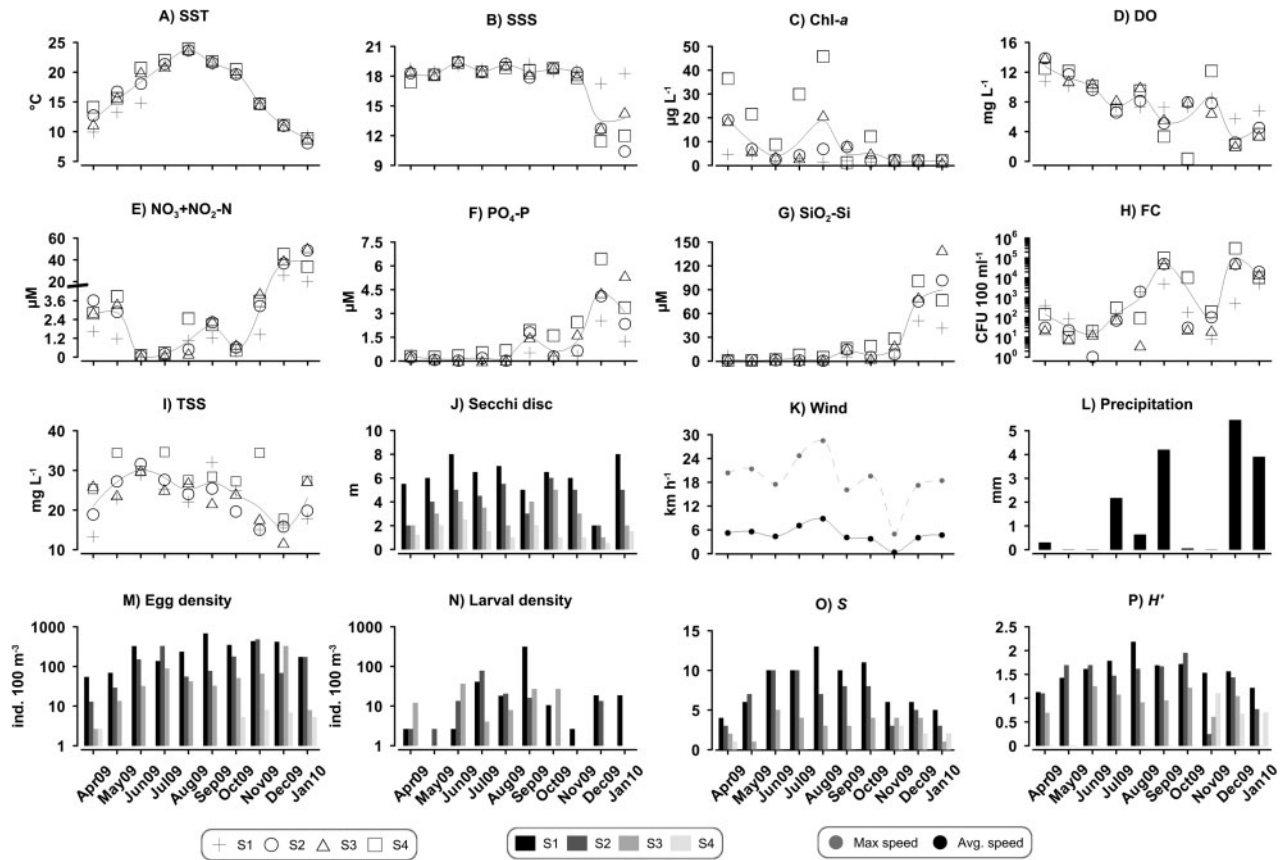


Figure 2. Monthly environmental variables of (a) SST, sea surface temperature; (b) SSS, sea surface salinity; (c) chl-*a*, chlorophyll *a*; (d) DO, dissolved oxygen; (e)  $\text{NO}_3+\text{NO}_2\text{-N}$ , nitrite and nitrate; (f)  $\text{PO}_4\text{-P}$ , ortho-phosphate; (g)  $\text{SiO}_2\text{-Si}$ , silicate; (h) FC, fecal coliform; (i) TSS, total suspended solid; (j) Secchi disc depth; (k) maximum and average wind speed; (l) average precipitation amount of 5 days prior to and on the cruise day; and estimated biological variables (m) fish egg density; (n) larval density; (o) *S*, species number; and (p)  $H'$ , diversity index at four sampling stations measured between April 2009 and January 2010 in the Golden Horn Estuary. The black solid lines (a–l) indicate the smoothed average values at four stations for each variable.

DO concentrations were highest in spring and lowest in winter while overall concentrations for regions were highest in April 2009 and lowest in December 2009 (Figure 2d).

Biochemical and microbiological characteristics showed significant seasonal changes with no distinct spatial pattern (Table I; Figures 2e–h). Overall, nutrient concentrations peaked in early autumn and again in winter, despite the remaining environmental parameters being at their minima.  $\text{NO}_3+\text{NO}_2\text{-N}$  concentrations reached approximately  $40 \mu\text{M}$  while  $\text{PO}_4\text{-P}$  concentration was  $4.5 \mu\text{M}$  and  $\text{SiO}_2\text{-Si}$  had a concentration of  $100 \mu\text{M}$  in December 2009 (Figures 2e–g). FC measurements showed two peaks in the sampling period (Figure 2h) with a high association with precipitation (Spearman rank  $R = 0.648$ ,  $P < 0.01$ ).

TSS measurements showed high seasonal fluctuations (Table I). The lowest values were found in winter (Figure 2i). Surprisingly, there was no correlation between TSS and precipitation ( $P > 0.05$ ). The greatest Secchi disc depth was measured in the

lower exit station S1, whereas the lowest was observed in the upper estuary station S4 (Figure 2j). Turbidity levels were higher throughout the year at S4 and reached their maximum values for all stations in December 2009, where rainfall was higher at  $5 \text{ mm}$  and wind speed was lower at  $4 \text{ km h}^{-1}$  (Figures 2j–l).

#### *Ichthyoplankton composition and density*

The fish eggs in the Golden Horn estuary belonged to 21 taxa. Of these, nine were identified to species level, eight to genus level and three to family level (Table II). The most abundant species were *Liza* sp. (12.7%), *Mullus* sp. (12.3%), *Diplodus* spp. (11.8%), *Scorpaena* sp. (8.5%) and *Gaidropsarus mediterraneus* (Linnaeus, 1758) (7.1%). Ten species – *G. mediterraneus*, *Merlangius merlangus* (Linnaeus, 1758), *Ctenolabrus rupestris* (Linnaeus, 1758), *Scomber* sp., *Serranus hepatus* (Linnaeus, 1758), *Pomatomus saltatrix* (Linnaeus, 1766), *Trachinus draco* Linnaeus, 1758, *Microchirus* sp., *Arnoglossus* sp. and Soleidae (sp.) – were found only as eggs. The fish larvae in

Table II. CCA code, spatial distribution and percentage (%) of the total density of fish eggs and larvae in the Golden Horn estuary between April 2009 and January 2010.

Family	Species	CCA code	Lower exit		Lower estuary		Middle estuary		Upper estuary	
			S1		S2		S3		S4	
			Egg	Larv	Egg	Larv	Egg	Larv	Egg	Larv
Gobiidae	<i>Pomatoschistus marmoratus</i> (Risso, 1810)	Pmar	–	0.1	–	–	–	0.6	–	–
Scorpaenidae	<i>Scorpaena</i> sp.	Scor	6.9	–	1.6	0.5	–	–	–	–
Clupeidae	<i>Sardinella aurita</i> Valenciennes, 1847	Saur	0.5	0.1	0.1	0.1	–	–	–	–
Clupeidae	<i>Sprattus sprattus</i> (Linnaeus, 1758)	Sspr	3.7	0.6	–	0.2	–	–	–	–
Engraulidae	<i>Engraulis encrasicolus</i> (Linnaeus, 1758)	Eenc	2.6	1.8	0.6	–	0.7	–	–	–
Sparidae	<i>Diplodus</i> spp.	Dipl	9.4	0.1	1.0	–	1.4	–	–	–
Mullidae	<i>Mullus</i> sp.	Mull	6.8	4.4	4.8	0.4	0.7	0.1	–	–
Serranidae	<i>Serranus hepatus</i> (Linnaeus, 1758)	Shep	1.9	–	0.5	–	–	–	–	–
Gadidae	<i>Merlangius merlangus</i> (Linnaeus, 1758)	Mmerl	2.7	–	1.1	–	2.8	–	–	–
Gadidae	<i>Gaidropsarus mediterraneus</i> (Linnaeus, 1758)	Gmed	5.3	–	1.1	–	0.7	–	–	–
Mugilidae	<i>Liza</i> sp.	Liz	1.0	–	8.5	0.5	3.0	–	0.2	–
Soleidae	<i>Microchirus</i> sp.	Micr	1.9	–	1.0	–	–	–	–	–
Labridae	<i>Ctenolabrus rupestris</i> (Linnaeus, 1758)	Crup	0.5	–	0.1	–	–	–	–	–
Trachinidae	<i>Trachinus draco</i> Linnaeus, 1758	Tdrac	1.0	–	0.3	–	–	–	–	–
Carangidae	<i>Trachurus</i> sp.	Trach	0.4	–	1.6	0.3	–	0.1	–	–
Pomatomidae	<i>Pomatomus saltatrix</i> (Linnaeus, 1766)	Psal	0.4	–	–	–	–	–	–	–
Bothidae	<i>Arnoglossus</i> sp.	Arno	1.5	–	0.5	–	0.5	–	–	–
Scombridae	<i>Scomber</i> sp.	Scom	0.2	–	–	–	–	–	–	–
Sparidae	Unidentified	Spar	0.3	0.2	–	0.0	0.7	0.6	–	–
Gobiidae	Unidentified	Gobi	–	0.0	–	0.5	–	0.6	–	–
Mugilidae	Unidentified	Mugi	–	–	1.2	0.0	0.4	0.1	0.3	–
Soleidae	Unidentified	Sole	1.0	–	0.2	–	–	–	–	–
Gadidae	Unidentified	Gadi	1.5	0.2	0.4	–	0.4	–	–	–
No fish	Unidentified	No fish	0.9	0.1	0.6	0.1	0.3	0.0	–	–

the estuary belonged to 13 taxa; four of these were identified to the species level, five to the genus level, and three to the family level (Table II). The most abundant fish larvae were *Mullus* sp. (4.9%) and *Engraulis encrasicolus* (Linnaeus, 1758) (1.8%). Lower estuary stations provided 86.1% of ichthyoplankton density and the highest proportion of density was determined in the lower exit station S1 with 57.8%. Values decreased strictly through the upper estuary and a very low (0.5%) density of fish eggs belonging to two species was detected at the upper estuary station S4, while no fish larvae were detected at that station (Table II). Density values showed significant differences among stations while no significant differences were found for seasonality (Table I). Fish eggs and larvae were most abundant in summer and autumn when species diversity ( $H'$ ) was also high. Surprisingly, only six taxa were found to have their lowest densities in spring. The largest densities of fish eggs and larvae were found in September 2009, with 786.4 ind. 100 m<sup>-3</sup> and 355.9 ind. 100 m<sup>-3</sup>, respectively (Figures 2m,n). For the fish larvae, data were only available for three stations because no fish larvae were sampled from S4 (Figure 2n) and density values showed differences between stations S1, S2

and S3 (Tukey HDS, d.f. = 27,  $P < 0.01$ ). Numbers of species ( $S$ ) and Shannon diversity ( $H'$ ) indices were highest in the summer season and in the lower estuary (Figures 2o,p). Similar to density values, significant regional differentiation in  $S$  and  $H'$  was observed, but no seasonal changes were detected (Table I). According to the post-hoc assumption of ANOVA results, values for  $S$ ,  $H'$  and egg densities (Tukey HDS, d.f. = 36,  $P < 0.05$ ) were different for stations S1, S3 and S4. The results of Spearman rank correlation showed that fish eggs and larvae,  $S$  and  $H'$  were positively correlated with depth and water clarity. Also, eggs and larval density showed negative correlation with chl-*a*. However, SST and SSS did not show any correlation with any biological variable (Table III).

Two main seasonal groups as 'summer–autumn' and 'autumn–winter' were indicated by the nMDS ordination of the fish egg samples (Figure 3). Three samples from S4 were distant from the others, indicative of a spatial segregation. Seasonal differences of the fish eggs component were evident in the summer–winter samplings (ANOSIM: Global  $R = 0.817$ ,  $P < 0.01$ ). A weak spatial grouping, separating the assemblages of S1, S2 and S3 with that of the upper estuary station S4, was evident in the

Table III. Results of Spearman rank correlations between environmental variables and biological components.

	Depth	SST	SSS	Chl- <i>a</i>	Secchi	NO <sub>2</sub> +NO <sub>3</sub> -N	PO <sub>4</sub> -P	SiO <sub>2</sub> -Si	FC	DO	TSS
Egg density	0.809*	–	–	–0.572*	0.698*	–	–	–	–	–	–0.451*
Larval density	0.577*	–	–	–	0.454*	–	–	–	–	–	–
S	0.724*	–	–	–0.510*	0.716*	–	–	–	–	–	–
H'	0.697*	–	–	–	0.663*	–0.405*	–0.518*	–	–	–	–

Environmental variables (depth; SST, sea surface temperature; SSS, sea surface salinity; Chl-*a*, chlorophyll *a*; Secchi, Secchi disc depth; NO<sub>2</sub>+NO<sub>3</sub>-N, PO<sub>4</sub>-P, SiO<sub>2</sub>-Si, nutrients; FC, fecal coliform; DO, dissolved oxygen; TSS, total suspended solid).

Biological components (fish egg density; larval density; S, number of species; H', diversity index).

\* $P < 0.01$ .

autumn–winter samples (ANOSIM: Global  $R = 0.204$ ,  $P < 0.05$ ; Figure 3a). This was mainly due to *Liza* sp. being present in all S4 samples during the winter with very low densities. *Mullus* sp. and *E. encrasicolus* dominated the summer samples and they were mainly found at the lower exit station S1 at high density values. The genus *Diplodus* was found year-round, and showed some degree of regional distribution, being less abundant through the upper part of the estuary. In addition to the seasonal assemblages, the monthly components within a season show some similarities to the following months. The group summer–autumn indicated high similarities within the months August and September 2009. The species mostly responsible for the similarities between these two months were *Scorpaena* sp., *E. encrasicolus* and *Arnoglossus* sp. Also, *Liza* sp., Gadidae and *Diplodus* spp. were responsible for the similarities of the months November and December 2009, which resulted in the autumn–winter group for the fish egg component. Likewise, the fish larvae showed two main seasonal groups, winter and summer (Figure 3b). Both seasonal and temporal differences of the fish larvae component were significant in summer–winter (ANOSIM: Global  $R = 0.777$ ,  $P < 0.01$ ) and

in S1 and S3 (ANOSIM: Global  $R = 0.405$ ,  $P < 0.05$ ; Figure 3b). Summer samples were dominated by *E. encrasicolus*, *Mullus* sp. and *Trachurus* sp. with high density values at S1. *Sprattus sprattus* (Linnaeus, 1758) was characteristic of the winter assemblage. *Liza* sp. is the main species responsible for spatial differences between S1 and S3 during the winter. Unlike the fish eggs, no fish larvae were detected in the upper estuary at station S4.

#### Relationship between larval assemblage and environmental variables

The Monte Carlo permutation test indicated that four environmental variables (depth, SST, chl-*a*, Secchi disc depth) were significantly ( $P < 0.05$ ) associated with the larval fish distribution. Therefore, CCA was performed for those four significant environmental variables over the density distribution of 13 larval fish species. The first two axes of the CCA showed statistical significance ( $P < 0.05$ ) and the species–environment correlations were found to be 0.989 and 0.751 for Axis 1 and Axis 2, respectively. The first two CCA axes represented 70.8% of the cumulative variance that explained the species–environment relationship (43.5% and 26.6% for Axis 1 and Axis 2, respectively). Axis 1 explained 43.5%

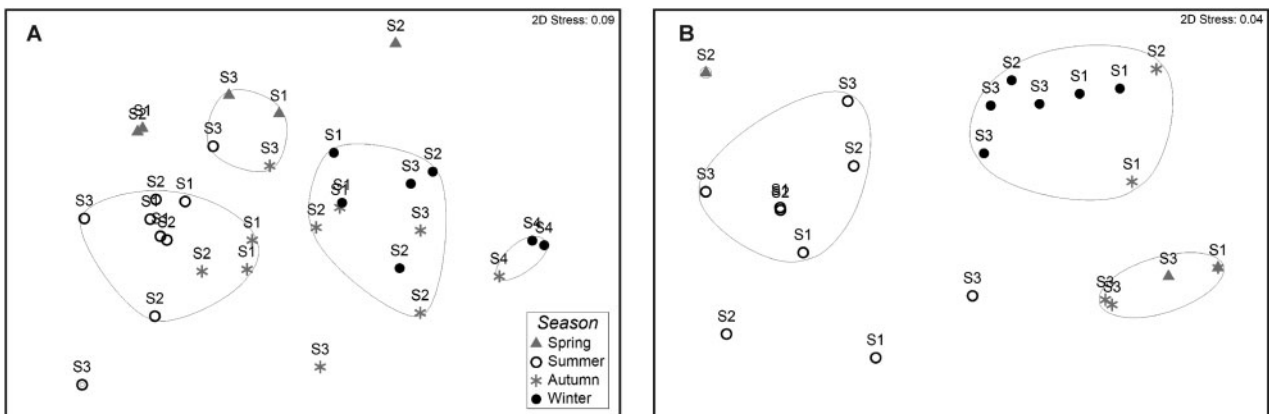


Figure 3. Non-metric multidimensional scaling (nMDS) plots of ichthyoplankton samples, showing the cluster of samples greater than 3% total density. (a) Fish eggs and (b) fish larvae in seasons (symbols) and in stations (labels). MDS plots generated from the Bray–Curtis similarity matrix using square-root transformed species density data.



of the variation in species density, and positively correlated with SST, chl-*a* and Secchi disc depth and negatively with depth. Axis 2 accounted for 26.6% of the variability explained, and positively correlated with depth, Secchi disc depth, SST and negatively with chl-*a* (Figure 4). The seasonal variation of species assemblages was tracked with Axis 1 of CCA while Axis 2 indicated regional variation. In this sense, lower estuary samples characterized by deep and less turbid waters were positioned in the upper part of the CCA bi-plot. On the contrary, shallow waters with low clarity and high chl-*a* values of the middle and upper estuary samples were located in the lower part of the CCA bi-plot. The upper right side of this plot indicated segregation of summer samples characterized by high temperature, high water clarity and chl-*a* values. Species such as *E. encrasicolus*, *S. aurita*, *Mullus* sp. and *Diplodus* spp. were directly related to high temperature and Secchi disc depth and were restricted to the middle and upper estuary region. The lower left side of the bi-plot showed middle estuary (S3) samples characterized by shallow depth and less clarity but high chl-*a* values. Species located in that space (*Liza* sp., Mugilidae (sp.)) were absent in the lower exit of the estuary (S1). *Scorpaena* sp., *Trachurus* sp., Sparidae (sp.) and Gobiidae (sp.) were positioned close to the centre of the ordination diagram and showed low or variable association with the environmental parameters of this CCA (Figure 4).

## Discussion

### *Anthropogenic influences on the hydrophysical conditions of the Golden Horn*

One of the main characteristics of estuaries is a sharp lateral salinity gradient (McLusky & Elliott 2004; Bianchi 2007). However, the SSS of the Golden Horn was similar to that of the Istanbul Strait upper layer (Altıok et al. 2014), where no significant lateral salinity gradient was detected. The reason behind this uniform SSS was the reduced freshwater input to the estuary after dam construction in the 1980s (Sur et al. 2002). Therefore, at present the main freshwater input into the Golden Horn is rainfall (Kor 1963), which manifests itself as sudden decreases in SSS during periods of high precipitation in winter. Chl-*a* and Secchi disc depth were the only parameters that showed significant regional differences. Chl-*a* values were mostly uniform in the lower part of the estuary, while two seasonal peaks during spring and summer were detected in the upper estuary. Taş et al. (2009) emphasized that high chl-*a* values can be explained by phytoplankton blooms such as those of the harmful algae species *Microcystis aeruginosa* (Kützing) Kützing (Taş et al. 2006) and *Prorocentrum cordatum* (Ostenfeld) Dodge (Taş & Okuş 2011). Water clarity, as inferred from Secchi disc depths, decreased sharply from the lower exit to the upper estuary (McLusky & Elliott 2004), and was affected by high wind speed and precipitation. Fluctuations and sudden increases in nutrient

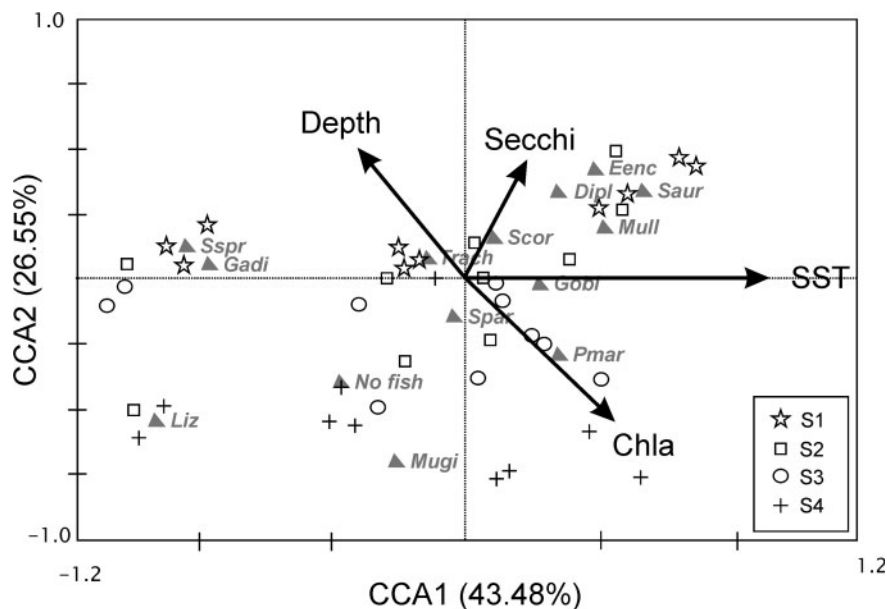


Figure 4. Canonical correspondence analysis (CCA) bi-plot ordination diagram of the larval fish assemblages in the Golden Horn estuary using the first two axes. Samples were classified in stations (S1, S2, S3, S4). Significant environmental variables are plotted as arrows (depth; Secchi, Secchi disc depth; SST, sea surface temperature; and Chla, chlorophyll *a*). Species codes are presented in Table II.

concentrations and the presence of FC were connected to low DO levels in winter. These results point to ongoing anthropogenic pollution despite the recent connection (in 2002) of the domestic discharges of the Golden Horn to a collector system to decrease pollution (Aslan-Yılmaz et al. 2004). Pollution might still be occurring via point sources, one of which (around station S3) was reported to be temporarily active due to problems with the collector system during the time of this and subsequent studies (Zeki 2012).

#### *Spatial and temporal patterns of ichthyoplankton dynamics*

One general characteristic of estuaries is a markedly seasonal abundance and diversity of fish species (McLusky & Elliott 2004). The Golden Horn, however, only partially reflected this general pattern. The ichthyoplankton assemblage of the Golden Horn estuary consisted of 23 species, similar to the previous study carried out in the region (Yüksek et al. 2006), and was dominated by *Mullus* sp., *Diplodus* spp. and *Liza* sp. Its composition showed some similarities to the temperate estuaries of the Mediterranean Sea (Akin et al. 2005; Elliott et al. 2007; Maci & Basset 2009) as well as to the coastal assemblage of the Sea of Marmara (Demirel et al. 2007) and the Aegean Sea (Demir 1969, 1974; Damalas et al. 2010; Somarakis et al. 2011). As supported by the multivariate analysis, most species showed a seasonal pattern with the presence of higher densities during the summer and winter. Moreover, the spatial pattern showed that ichthyoplankton distribution and diversity was relatively high in the lower part of Golden Horn and gradually decreased through the upper estuary. The results of the correlation analysis showed that the upper estuary was preferred less by adult fish for spawning due to less water clarity, high chl-*a* and TSS values. The temporal changes of early life stages were directly related to the spawning strategy and preference of adult fish (Murua et al. 2003; Miller & Kendall 2009). Thus, there were high densities of *Mullus* sp., *Scorpaena porcus* Linnaeus, 1758, *Engraulis encrasicolus*, *Trachurus* sp. and *Microchirus* sp. during the summer–early autumn period, while there were high densities of *Sprattus sprattus*, *Merlangius merlangus*, *Liza* sp. and *Gaidropsarus mediterraneus* during late autumn–winter. The families Clupeidae, Mullidae, Mugilidae and Gadidae dominated the ichthyoplankton composition, exhibiting high mutuality for the northeast coasts of the Sea of Marmara (Alimoğlu 2002). The densities of flatfish and wrasse eggs were very low and belonged to four species only: *Microchirus* sp., *Arnoglossus* sp., Soleidae (sp.)

and *Ctenolabrus rupestris* (Linnaeus, 1758). In addition, early life stages of other typical coastal demersal fishes such as gurnard and stargazer were absent in the samples. The low numbers of the early stages of flatfish, goby, gurnard and stargazer may be due to the poor sediment quality. For example, recent studies postulated the heavy metal loads and increasing levels of organic carbon, iron, lead and mercury in surface sediments (Erşan et al. 2009) may have compromised the benthic infaunal communities of the estuary (Albayrak et al. 2010). However, sediments were not sampled to test this possibility.

#### *Larval assemblage of the Golden Horn*

Spatial changes in water depth and clarity were the main factors forcing larval assemblage distribution, leading to a decrease in density and diversity of fish larvae through the upper part of the estuary. Marine fish larvae are visual feeders and their feeding activity mostly occurs during daylight (Lasker 1981). Hence, the feeding success of fish larvae might be low in the less-clear waters of the Golden Horn estuary. Spatial variations in environmental parameters appeared to affect the assemblage structure and distribution. For the seasonal changes, SST and chl-*a* were the main factors in shaping the structure of larval assemblages and increasing SST, leading to increased density and diversity of fish larvae. Also, chl-*a* values reached their maxima during the summer in the upper estuary region. However, in addition to larval density showing a positive relationship with chl-*a*, a negative relationship was observed on a regional basis where the values were highest. The spawning activity of many fishes occurs at the same time as phytoplankton blooms (James et al. 2003; Platt et al. 2003). This synchronization and the following zooplankton bloom support the successful first-feeding of their larvae (Otterå et al. 2006). However, an adverse condition for the larvae can be the harmful algal blooms which are regularly reported during the April–September period in the upper part of the Golden Horn (Taş & Okuş 2011). The larval and juvenile stages of fish are vulnerable to the dinoflagellate toxins. If the planktonic food web is affected by algal toxin, this may cause a significant reduction of larval survival rate (Gosselin et al. 1989). This could, in turn, influence estuarine communities at different trophic levels, because the very few studies on the mesozooplankton composition of the Golden Horn pointed out that marine zooplankton species were present at the estuary, but their total density and species diversity patterns were significantly lower than at the other coastal areas of the Sea of Marmara (Dorak 2010).

Elliott & Hemingway (2002) emphasized that the functional groups of fish species that use an estuary as a nursery demonstrate the healthiness of the estuary. According to the category of life strategies of fish (Franco et al. 2008), estuarine species were the least frequently represented group in this study. Their densities were low and distributions were restricted in the estuary. *Pomatoschistus marmoratus* (Risso, 1810), *Liza* sp. and Gobiidae were the only estuarine species observed in the middle parts, with similar density values to the lower parts. *Pomatoschistus marmoratus* is a very common species found in coastal areas of the Sea of Marmara, where there is a high influence of freshwater. This species did seem not to prefer the Golden Horn as a nursery area. Furthermore, larvae of *Scorpaena* sp. were collected only in the lower part, despite the fact that it is classified as an estuarine species and it has a high density of adults around the coast of Istanbul. The restricted larval distribution of those species may be caused by the altered salinity of the Golden Horn. It was asserted that estuaries with similar geomorphologies but different human impact could maintain distinct larval assemblages (Ramos et al. 2012). For example, the spatio-temporal distribution of salinity in relation to larval fish assemblage can be a good predictor of larval distribution (Chiappa-Carrara et al. 2003; Martino & Able 2003). In addition to altered salinity, high nutrient loads and microbiological contamination could be responsible for a low environmental status and lead to a decrease in larval diversity in estuaries (Ramos et al. 2012).

## Conclusion

The present study showed that environmental conditions in the Golden Horn estuary gradually deteriorated towards the upper part. Compared to the lower estuary, excessive chlorophyll-*a* values, sudden increases in nutrients and fecal coliforms associated with low dissolved oxygen levels and low water clarity were observed seasonally in the upper parts. Contrary to these gradients, there was no horizontal salinity gradient in the estuary due to the reduced freshwater inputs due to dam construction. Thus there was uniform surface salinity from the lower to the upper part of the estuary. Although this study covered a 10-month period, it showed a clear spatio-temporal distribution of ichthyoplankton density and diversity values. Larvae exhibited two distinct seasonal assemblages (winter and summer) and two distinct regional assemblages (lower and middle estuary). Fish eggs and larvae mainly aggregated in the lower part but were not observed in the upper part. Finally, this study revealed that in the Golden Horn estuary, the

pelagic fish larvae are sensitive to environmental conditions and the structure of the larval assemblage is shaped by several environmental variables. Future studies should include extended and frequent samplings from the exit to the sea to the stream mouth, which would provide a more detailed insight into the dynamics of the larval fish community.

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