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Genetic Identification of Ichthyoplankton in the Black Sea and Their Abundance and Community Assemblages

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Abstract

Accurate identification of fish species during the planktonic stages is vital for detecting spawning, foraging, and growth sites of fish species to provide data for stock assessment, environmental impact assessment, and ecological monitoring. In this study, seasonal ichthyoplankton abundance, community composition, and main possible environmental drivers that influence their community composition were investigated in the Southeastern Black Sea. Ichthyoplankton samples were seasonally collected for a year from four stations, two coastal and two offshore sites. DNA barcoding method was applied to identify ichthyoplankton specimens. Of 33 ichthyoplankton taxa identified using DNA barcoding, 31 taxa were identified at species level and 2 taxa were identified at genus level due to unavailability of reference sequences from the Black Sea in public databases. *Engraulis encrasicolus*, *Sprattus sprattus*, *Mullus barbatus*, and *Trachurus mediterraneus* were identified as the most abundant species. Engraulis encrasicolus alone accounted for nearly 91.5% of the total catch. Seasonality had a strong influence on the ichthyoplankton assemblages. The highest ichthyoplankton abundance was found in summer, followed by winter, autumn, and spring. While 30 fish taxa were identified in summer, 10 taxa were identified in spring, 3 taxa were identified in both autumn and winter. Ichthyoplankton communities were dominated by *E. encrasicolus*, *M. barbatus*, and *T. mediterraneus* specimens in summer, *S. sprattus*, and *M. merlangus* specimens in winter, *S. sprattus* and *G. mediterraneus* specimens in autumn, *Alosa immaculata* and *Merlangius merlangus* in spring. The ichthyoplankton abundance and richness was generally higher in coastal stations in each season. *Engraulis encrasicolus*, *S. sprattus*, *M. merlangus*, *P. incognitus* were the only species that detected in more than 4 hauls. On the other hand, *M. batrachocephalus*, *P. flesus*, *S. abester*, *T. draco*, *U. cirrosa*, and *C. lucerna* were only detected in a single haul. Overall, the dominant environmental variable affecting abundance of ichthyoplankton was temperature. The relationship between community composition and environmental variable based on nMDS analysis indicated that ichthyoplankton assemblage structure is also influenced by salinity, dissolved oxygen, chlorophyll-a, NO2, and NO3.

Introduction

The ichthyoplanktonic stage is a life stage of the fish that is found most abundantly in the marine environment (Lewis et al., 2016). Correct identification of fish during the ichthyoplanktonic stage is vital for detecting spawning and foraging sites of fish species. Hence, there are numerous ichthyoplankton monitoring programs worldwide to provide data for ecological monitoring (Şahin & Düzgüneş, 2019), environmental impact assessment (Coker & Cihangir, 2018), stock assessment (Richardson et al., 2010), and establishing marine protected areas (Guyah et al., 2021). The traditional identification of ichthyoplankton has been based on morphological diagnostic characters such as egg shape, egg size, body shape, pigmentation, meristic count and measurements (Satilmis et al., 2014; Şahin & Düzgüneş, 2019; Klimova et al., 2021). However, the number of discriminative morphological features are sometimes not enough to make accurate identification

at the species or at the genus level, especially in species-rich families (Hubert et al., 2010). The main limitations of morphological identifications are intra-species variation, ontogenetic variation, loss of external pigmentation due to applied preservation techniques, and loss of discriminative body parts (Ko et al., 2013; Becker et al., 2015; Hubert et al., 2015). Intensive labor is required to process bulk samples and to isolate fish larvae and eggs. Moreover, it is not always possible to make identification at the species level based on morphological characters (Ko et al., 2013; Becker et al., 2015; Lewis et al., 2016; Azmir et al., 2017). The use of molecular tools for species identification opened a new perspective in ichthyoplankton studies and is proven to improve species identification accuracy (Wibowo et al., 2015; Collet et al., 2018; Panpromnin et al., 2020). DNA barcoding, identification based on cytochrome c oxidase I (COX1) sequence, is the most commonly applied molecular tool for species identification (Herbert et al., 2003; Aydın & Öztürk, 2021; Karadurmuş et al., 2022; Öztürk et al., 2022). In ichthyoplankton studies, DNA barcoding allows identification of species having ontogenetic changes and lacking diagnostic morphological characters with high accuracy (Bucklin et al., 2010; Ko et al., 2013; Frantine-Silva et al., 2015; Hubert et al., 2015; Azmir et al., 2017).

Numerous studies focused on the identification, distribution, and community composition of ichthyoplankton in the Black Sea. To date, all published studies regarding the identification of ichthyoplankton in the Black Sea have been based on morphological characters. Selifonova (2012) assessed the taxonomic composition and abundance of ichthyoplankton off the Novorossiysk and Tuapse coasts (the northeastern Black Sea) and reported presence of 33 taxonomic groups. Satilmis et al. (2014) assessed the seasonal changes in ichthyoplankton assemblages off the coast of Sinop (the southern Black Sea) and 32 taxonomic groups were identified. Klimova and Podrezova (2018) studied seasonal distribution of the ichthyoplankton near the Crimean Peninsula (northern Black Sea) between 2011 and 2016, of which 40 taxonomic groups were identified. Klimova et al. (2021) assessed the distribution and composition of ichthyoplankton in spring and summer off the Crimean coast and 24 taxonomic groups were identified. Şahin and Düzgüneş (2019) assessed spatial and temporal variation of ichthyoplankton off the coast of Giresun (the southeastern Black Sea) and 26 taxonomic groups were identified.

Information on ichthyoplankton assemblages can be used to infer changes in fish community composition and abundance at the ecosystem level (Hernandez et al., 2010). Ichthyoplankton assemblages in coastal and offshore waters are complex in terms of species composition and their distribution and influenced by many factors. The spawning season of adult fish (Hernández-Miranda et al., 2003), egg dispersal (Öztürk & Altınok, 2021), larvae behavior (Hare & Govoni, 2005), oceanographic processes and environmental factors (Harris et al., 1999; Auth, 2008) result in spatial and temporal variation in abundance and composition. Changes in ichthyoplankton abundance and composition can be an early indicator of overexploitation and climate-related shifts.

This study is aimed to assess the abundance, distribution, and seasonal variation in ichthyoplankton composition in the southeastern Black Sea and their identification through DNA barcoding technique. This study also aimed to advance the knowledge of the influence of environmental conditions on ichthyoplankton abundance and composition.

Materials and Methods

Sampling

Ichthyoplankton samples were seasonally collected (8-9 November 2018, 1-2 February 2019, 2-3 May 2019, and 2-3 August 2019) at 4 stations: two coastal sites; K1 (41°00'03.6"N 40°13'19.2"E), K3 (41°10'01.2"N 40°42'43.2"E), and two offshore sites; K0 (41°35'13.2"N 40°21'28.8"E), K2 (41°16'51.6"N 40°12'03.6"E), on *KTU Denar-I R/V* in the Southeastern Black Sea (Figure 1). Sampling was carried out with a plankton net (0.5 x 0.5 m mouth opening, 4 m length, and 330 µm mesh size) equipped with a mechanical flowmeter for measuring the amount of water filtered. The nets were towed at the sea surface for 5 minutes at a speed of 2.5 knots. Bulk samples were preserved in 95% ethanol and kept at -20°C during sampling.

Environmental Parameters

Surface water temperature, salinity, dissolved oxygen, and conductivity were measured with Seabird SBE-37-SMP-ODO (Seabird Scientific, WA, USA). Chlorophyll-*a* (Chl-*a*), Ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), silicate (SiO_4), and phosphate (PO_4) concentrations in surface water were determined based on spectrophotometric methods (Bendschneider and Robinson 1952; Mullin and Riley 1955; Solorzano 1968; Cline 1969) by using a visible spectrophotometer (Shimadzu UV 2550, Japan). Total organic carbon (TOC) was measured based on the combustion catalytic oxidation method (Sharp 1973).

Ichthyoplankton Identification

A 2-stage sorting procedure was designated to retrieve as many ichthyoplankton as possible out of the bulk samples. Sorting procedures were performed under a dissecting microscope (Leica MZ60). In the first stage, fish eggs and larvae were sorted out from the bulk samples. In the second stage, fish eggs and larvae were identified to the lowest possible morphologically distinct taxon, herein morpho-species, based on diagnostic morphological characters, using the “look-

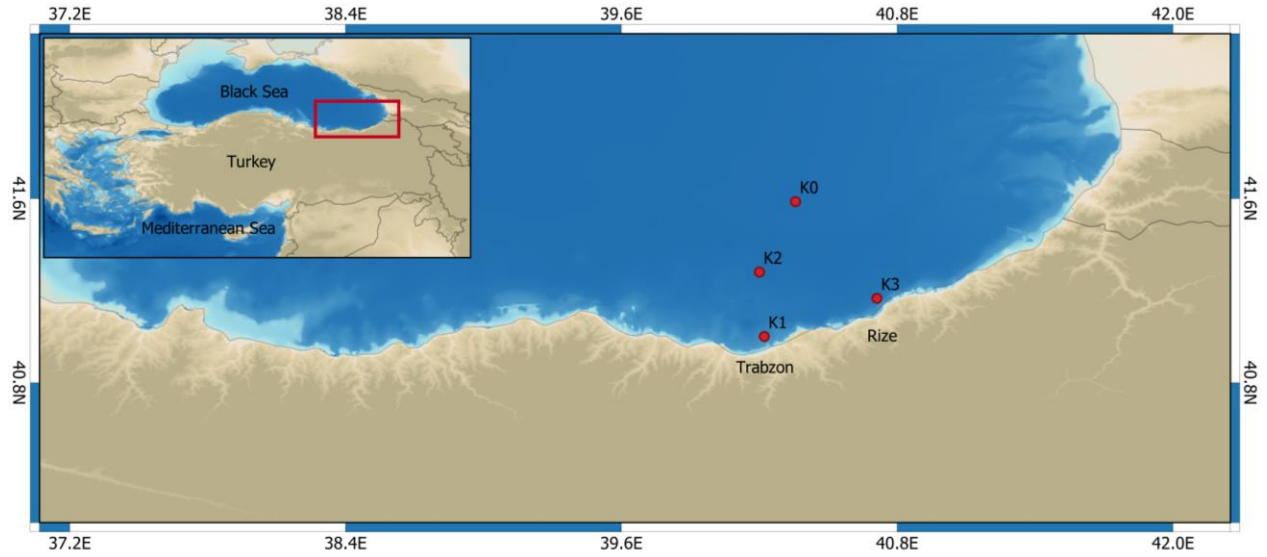


Figure 1. Ichthyoplankton sampling statins in the southeastern Black Sea. The map was generated in QGIS 3.16.

alike" method, described in Rodriguez et al. (2017) and Richards (2005). The number of individuals per morpho-species was recorded. Identified morpho-species were placed in labeled vials and preserved in 95% ethanol at +4°C.

The total number of collected eggs and larvae exceeded our processing capacity. To reduce cost and standardize the number of barcoded samples, up to six specimens of each larva and egg morpho-species (Table S1) were randomly selected for DNA barcoding.

Genomic DNA was extracted from larvae with the Wizard SV Genomic DNA Purification kit (Promega, USA) following the manufacturer's instructions. Prior to extraction, samples were hydrated with sterile ultra-pure water to improve digestion. The mitochondrial gene region of cytochrome oxidase subunit I (COX1) was targeted for genetic identification and characterization. COX1 gene region was amplified with the primers of Fish-F1 (5´-TCA ACC AAC CAC AAA GAC ATT GGC AC-3´) and Fish-R1 (5´-AGA CTT CTG GGT GGC CAA AGA ATC A-3´) (Ward et al., 2005). PCR was carried out with a total volume of 25 µl that contained 12.5 µl 2X Master Mix (Hibrigen, Turkey), 1 µM of each primer (10 pmol), 1 µl template DNA (80-150 ng), and 9.5 µl ultra-pure water. Amplifications were carried out in T100 thermal cycler (Bio-Rad, USA). The thermal program consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 50 s, annealing at 55°C for 45 s, extension at 72°C for 45 s, and followed by a final extension at 72°C for 5 min. Amplicons were visualized on a 1% agarose gel through staining of DNA-binding dye (Red safe). Standard ethanol precipitation was used to purify amplicons. Samples were bidirectionally sequenced using the BigDye Terminator Cycle Sequencing Kit v.3.1 on ABI 3500 Genetic Analyzer (Applied Biosystems, USA).

Chromatograms were controlled and checked manually. The raw sequences were edited and assembled using BioEdit v 7.2.5 (Hall, 1999). The Clustal W algorithm was used for sequence alignment. To detect possible artifacts and pseudogenes, gene regions were translated to protein sequences and compared with consensus protein sequences. The quality-checked sequences were compared with existing data in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank) using the BLAST function and the BOLD database (https://www.boldsystems.org/). A cutoff value for the BLAST results in the GenBank database was established as follows: query coverage >97% and identity >99.2% to perform identification at the species level. In the BOLD database, BIN analysis with the closest matching BIN (within 3%) and tree-based identifications were performed. Generated sequences were deposited in GenBank (Table 1).

Data Analysis

The abundance of ichthyoplankton was standardized and expressed as the total number of individuals per 100 m^3. All statistical analyses were conducted in R Studio (R Studio Team, 2020) using the *vegan* package (Oksanen et al., 2020). The data was visualized using *ggplot2* package (Wickham, 2016). The sampling map was generated in QGIS 3.16 (QGIS Development Team, 2021). For the community analysis, ichthyoplankton abundance data at the species level was hellinger transformed. Environmental parameters were standardized to z-scores. Seasonal and spatial variation patterns in the community composition of ichthyoplankton were assessed with non-metric multidimensional scaling (nMDS) based on the Bray-Curtis similarity measure using the "metaMDS" function. Possible seasonal and spatial differences in community composition were checked with the permutational multivariate analysis of variance (PERMANOVA) and Tukey HSD test was applied for pairwise comparison. The similarity percentage analysis (SIMPER) was performed using the "simper" function to

Table 1. Sampling stations, coordinates, season of the genetically identified species and their genbank accession numbers

Species	Order	Family	Season	Station	Coordinates	Gen Bank Accession
Aidablennius sphynx	Blenniiformes	Blenniidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148123-OK148126
Alosa immaculata	Clupeiformes	Clupeidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148129-OK148131
Atherina boyeri	Atheriniformes	Atherinidae	Summer Spring	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148133-OK148137
Belone belone	Beloniformes	Belonidae	Summer Spring	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148138-OK148145
Chelidonichthys lucerna	Perciformes	Triglidae	Summer	K1	41.001 N, 40.222 E	OK275348
Chelon saliens	Mugiliformes	Mugilidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148180-OK148183
Chromis chromis		Pomacentridae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148457-OK148460
Dicentrarchus labrax	Perciformes	Moronidae	Summer	K3	41.167 N, 40.712 E	OK148184
Diplodus annularis	Perciformes	Sparidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148185-OK148188
Engraulis encrasicolus	Clupeiformes	Engraulidae	Summer Spring	K0 K1 K2 K3	41.587 N, 40.358 E 41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK148603-OK148612
Gaidropsarus mediterraneus	Gadiformes	Lotidae	Autumn Winter	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148201-OK148206
Gobius niger	Gobiiformes	Gobiidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK463515-OK463516
Gobius paganellus	Gobiiformes	Gobiidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK463518-OK463519
Liza aurata	Mugiliformes	Mugilidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148189-OK148192
Merlangius merlangus	Gadiformes	Gadidae	Summer Spring Autumn	K1 K2 K3	41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK148194-OK148200
Mesogobius batrachocephalus	Gobiiformes	Gobiidae	Summer	K3	41.167 N, 40.712 E	OK463517
Mullus barbatus	Perciformes	Mullidae	Summer	K0 K1 K2 K3	41.587 N, 40.358 E 41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK148207-OK148211
Neogobius melanostomus	Gobiiformes	Gobiidae	Summer Spring	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148212-OK148216
Parablennius incognitus	Blenniiformes	Blenniidae	Summer Spring	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148218-OK148223
Pegusa sp.	Pleuronectiformes	Soleidae	Summer	K1 K2 K3	41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK156491-OK156493
Platichthys flesus	Pleuronectiformes	Pleuronectidae	Summer	K3	41.167 N, 40.712 E	OK148314
Salaria sp.	Blenniiformes	Blenniidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK156468-OK156469
Sarda sarda	Scombriformes	Scombridae	Summer	K1 K2 K3	41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK149204-OK149208
Sciaena umbra	Acanthuriformes	Sciaenidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148316-OK148319
Scophthalmus maximus	Pleuronectiformes	Scophthalmidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148320-OK148321
Scorpaena porcus	Scorpaeniformes	Scorpaenidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148323-OK148324
Spicara flexuosa	Perciformes	Centracanthidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148431-OK148434
Sprattus sprattus	Clupeiformes	Clupeidae	Autumn Winter	K0 K1 K2 K3	41.587 N, 40.358 E 41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK148556-OK148565
Symphodus roissali	Labriformes	Labridae	Summer Spring	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148514-OK148515
Syngnathus abaster	Syngnathiformes	Syngnathidae	Autumn	K1	41.001 N, 40.222 E	OK148614-OK148615
Trachinus draco	Trachiniformes	Trachinidae	Summer	K3	41.167 N, 40.712 E	OK148555
Trachurus mediterraneus	Carangiformes	Carangidae	Summer	K0 K1 K2 K3	41.587 N, 40.358 E 41.001 N, 40.222 E 41.281 N, 40.201 E 41.167 N, 40.712 E	OK148586-OK148591
Umbrina cirrosa	Acanthuriformes	Sciaenidae	Summer	K1	41.001 N, 40.222 E	OK148584
Uranoscopus scaber	Trachiniformes	Uranoscopidae	Summer	K1 K3	41.001 N, 40.222 E 41.167 N, 40.712 E	OK148598-OK148599

detect the species contributing to the seasonal and spatial community differences. The species with the highest contribution to community differences and environmental parameters were visually represented on nMDS ordination by using the "envfit" function.

Results

Environmental parameters

Seasonally, water temperature and salinity differed between 9.58°C-26.36°C and 14.16 ppt-18.29 ppt, respectively. Spatially, water temperature and salinity were similar among the sampling sites. The lowest dissolved oxygen concentrations (between 7.22-10.21 mg/l) were recorded in summer whereas the highest concentrations were recorded in winter (between 9.61-15.21 mg/l). Seasonal and spatial differences in water temperature, salinity, chlorophyll-*a*, NH_3, NO_2, NO_3, dissolved oxygen, PO_4, sigma-t, and TOC are visualized in Figure 2.

Ichthyoplankton Identification

A total of 29660 individuals, including 28308 eggs and 1352 larvae were collected throughout the study. 1352 larval samples were assigned into 36 morpho-species by morphological identification (Table S1). Whereas, among the 28308 egg samples 28 morpho-species were assigned by the morphological identification. A total of 304 specimens (160 larvae, 144 eggs) were subsampled for genetic species identification through DNA barcoding of which 93.75% (285 specimens) were successfully sequenced and analyzed. The size of the amplified products ranged between 491 and 686 bp. Of the analyzed remaining 285 specimens,

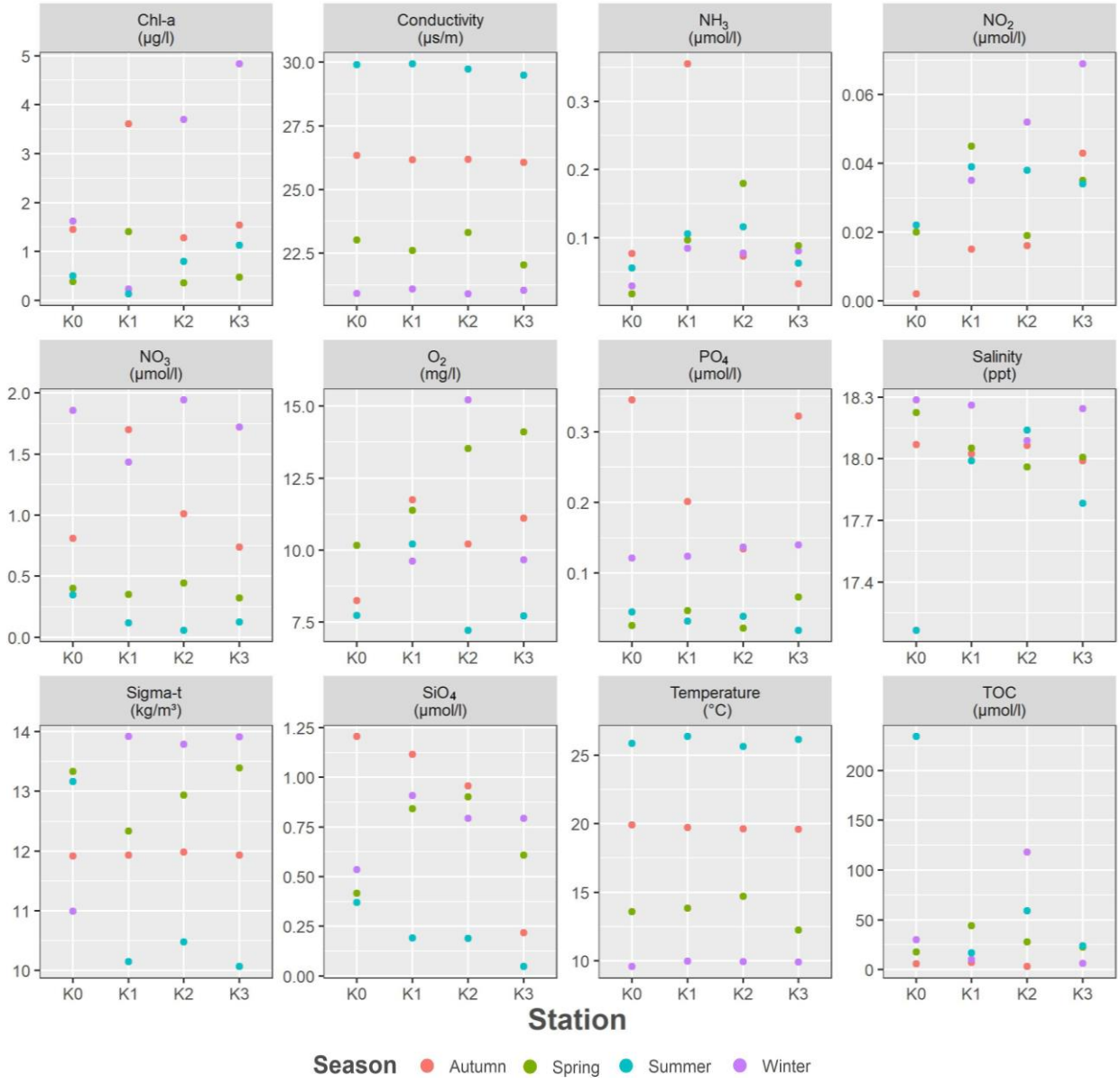


Figure 2. Environmental parameters.

33 fish species, belonging to 25 families were identified. While 23 fish species were identified both at larval and egg stages, 9 species were identified only at larval stage and a single species was identified only at egg stage.

Species richness was highest in the summer and lowest in the winter. In summer, 30 taxa were identified with a mean abundance of 4230±1816 ind.100 m^{-3}. In spring, 10 taxa were identified with a mean abundance of 31±16 ind.100 m^{-3}. In autumn, 3 taxa were identified with a mean abundance of 35±2 ind.100 m^{-3}. In winter, 3 taxa were identified with a mean abundance of 106±31 ind.100 m^{-3}. The detailed list of identified taxa and their Gen Bank accessions are presented in Table 1.

Community Composition

Among the samples collected during the ichthyoplankton surveys (n=16), ichthyoplankton was not detected in K0 and K2 stations in spring and autumn. The ichthyoplankton community was dominated by *Engraulis encrasicolus* (Linnaeus, 1758), *Merlangius merlangus* (Linnaeus, 1758), *Sprattus sprattus* (Linnaeus, 1758), *and Trachurus mediterraneus* (Steindachner, 1868) (Figure 3a). *Engraulis encrasicolus* constituted 91.5% of the ichthyoplankton community (Figure 3b). *Engraulis encrasicolus, S. sprattus, M. merlangus, Parablenius incognitus* (Bath, 1968) were the only species detected in more than 4 samples. Whereas *Mesogobius batrachocephalus* (Pallas, 1814), *Platichthys flesus* (Linnaeus, 1758), *Syngnathus abaster* (Risso, 1826), *Trachinus draco* (Linnaeus, 1758), and *Umbrina cirrosa* (Linnaeus, 1758) were seen only in one sample (Figure 3c). Changes in ichthyoplankton abundance in each season are presented in Figure 3d. Seasonally, the highest ichthyoplankton abundance was found in summer followed by winter and autumn. In contrast, the ichthyoplankton abundance in spring was the lowest. There was a significant difference in seasonal ichthyoplankton community composition (R^2=0.6941, F=13.61, P=0.01) between winter and the rest of the year. While *E. encrasicolus, Mullus barbatus* (Linnaeus, 1758), and *T. mediterraneus* specimens dominated the ichthyoplankton community in summer, *S. sprattus* and *M. merlangus* specimens dominated the ichthyoplankton community in winter. Whereas *S. sprattus* and *Gaidropsarus mediterraneus* (Linnaeus, 1758) specimens dominated the ichthyoplankton community in autumn. The ichthyoplankton abundance was generally higher in coastal stations (K1 and K3) in each season (Figure 3e). Yet, spatially, no significant difference was detected in terms of ichthyoplankton community composition (R^2=0.0901, F=0.59, P=0.8). While majority of the ichthyoplankton specimens were composed of eggs (Figure 3f), there was no significant difference between ichthyoplankton types in terms of community composition (R^2=0.0093, F=0.18, P=0.95). The majority of the egg specimens belonged to *E. encrasicolus* (>94%).

Non-metric multidimensional scaling revealed similarity in ichthyoplankton community composition between stations (Figure 4a) and similarity based on life stage (Figure 4b). In contrast, seasonal dissimilarities

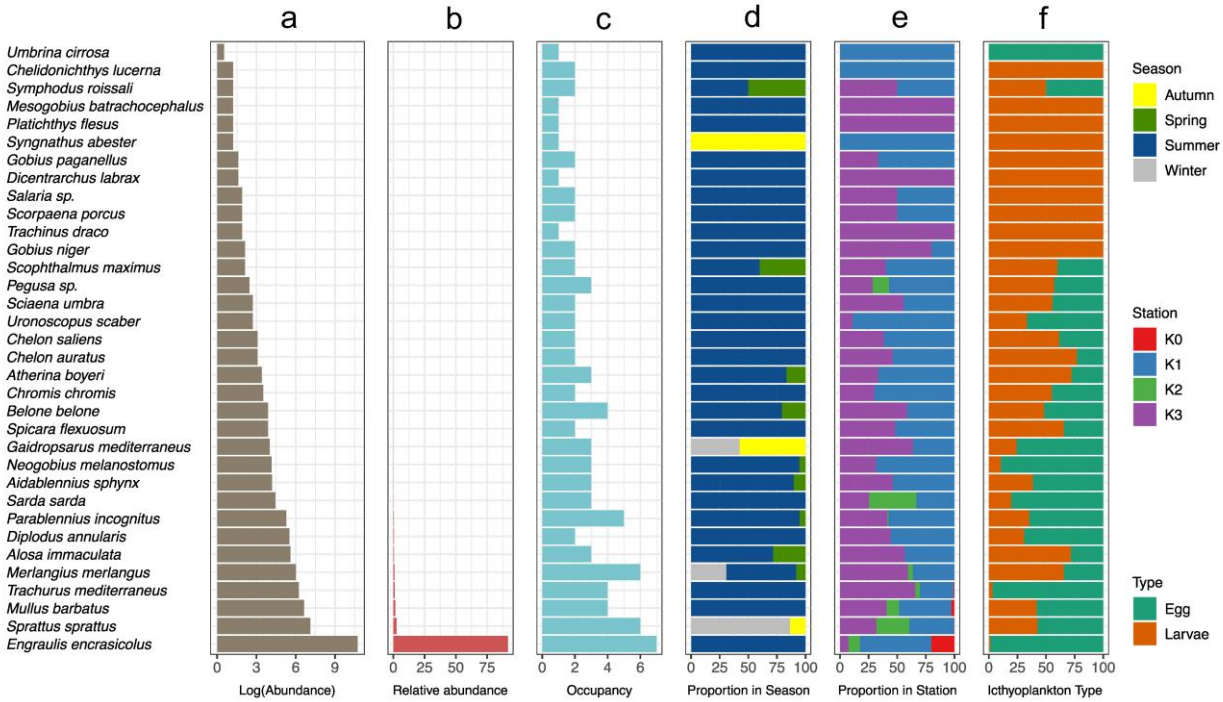


Figure 3. Taxonomic distributions of ichthyoplankton at the species level. Log-transformed number of detected specimens (a), relative abundance (b), occupancy (c) which is expressed as the number of stations (16 in total) in which the presence of a species detected, distribution of species across seasons (d), stations (e), and ichthyoplankton type (life stage) (f).

were observed (Figure 4c). The permutation test indicated that temperature, oxygen, salinity, NO_3, PO_4, and Chlorophyll-*a* significantly correlated with seasonal ichthyoplanktonic community composition. In contrast, NH_3, NO_2, SiO_4, TOC, and conductivity did not have any significant impact (P>0.05) on community composition. Among the significant environmental factors, temperature (r^2=0.674) was the most influential factor on ichthyoplanktonic community composition (Figure 5a).

SIMPER analysis revealed that increased abundance of *E. encrasicolus*, *S. sprattus*, *M. merlangus*, *G. mediterraneus*, and *A. immaculata* were the largest contributors to the dissimilarities between seasons. While *E. encrasicolus* and *S. sprattus* were the main contributors to the dissimilarity between winter and summer, *M. merlangus*, *G. mediterraneus*, and *A. immaculata* were the main contributors to the dissimilarity between autumn and spring (Figure 5b).

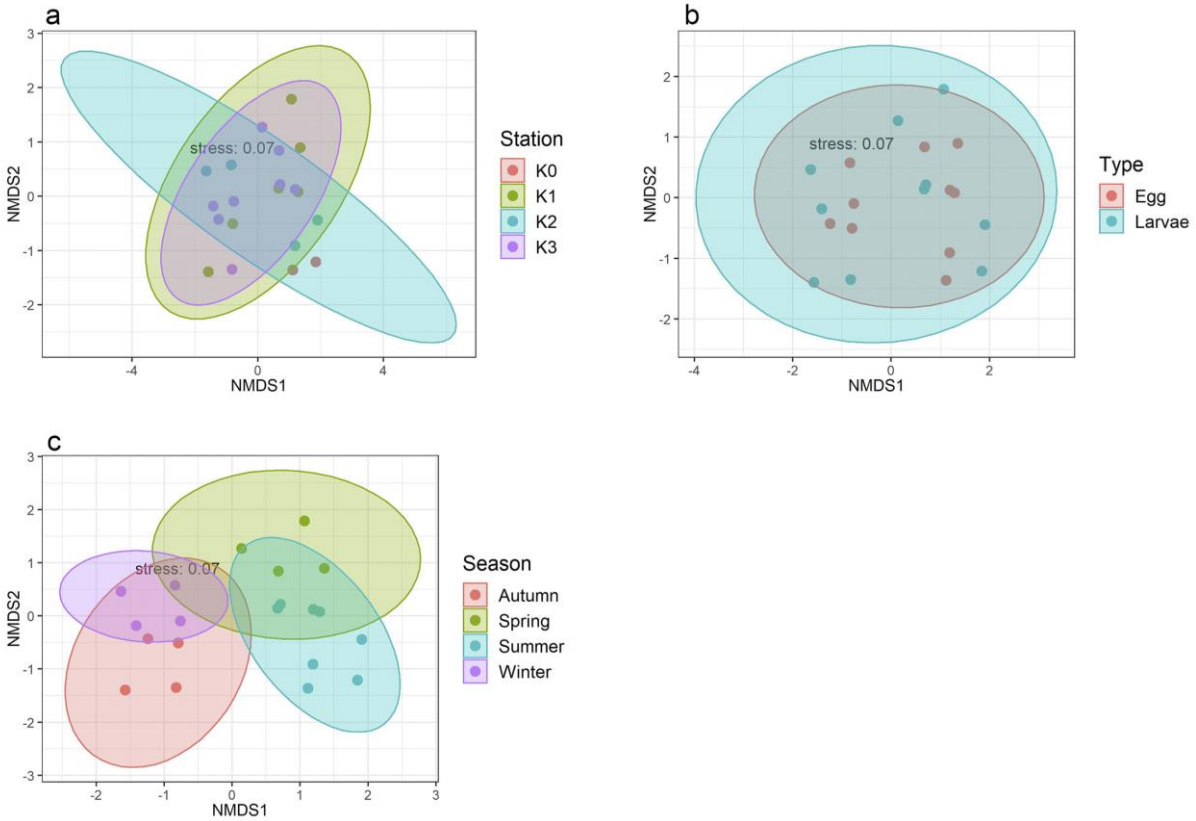


Figure 4. Two-dimensional Nonmetric multidimensional scaling (nMDS) ordination of ichthyoplankton communities based on station (a), type (b), and season (c).

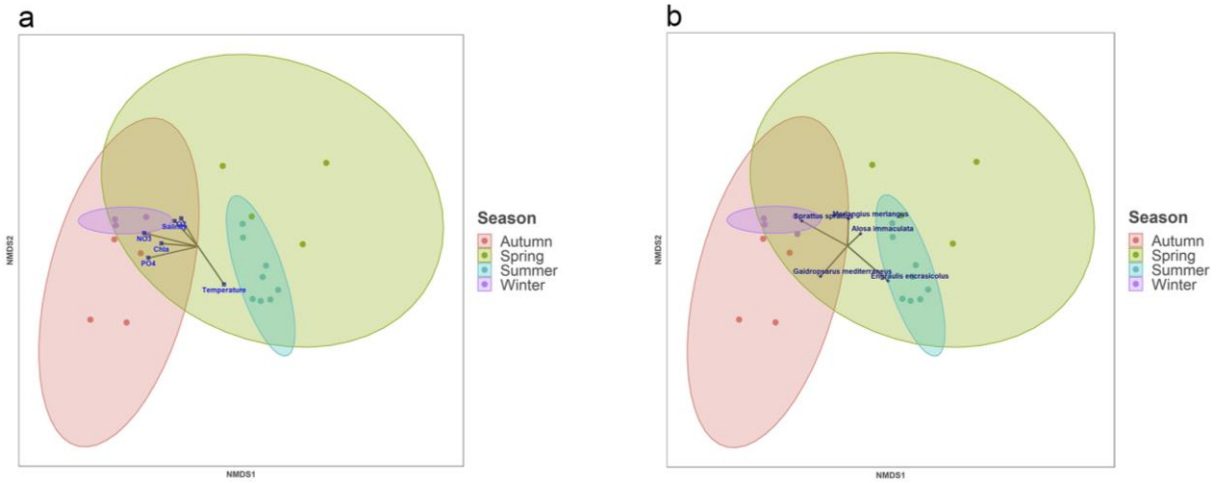


Figure 5. Two-dimensional Nonmetric multidimensional scaling (nMDS) ordination of ichthyoplankton communities based on season. Black lines represent environmental factors (a) and species (b) causing the significant differences in community composition. Strong predictors have longer lines than weak predictors.

Discussion

Although numerous ichthyoplankton surveys have been conducted in the Black Sea, only a handful of them has been conducted with a seasonal resolution and all of them relied on morphological identification methods. As many as 40 species were identified in a single study with a high spatial and temporal resolution (Klimova and Podrezova, 2018). In the present study, 33 taxa (31 at the species level) were identified with a seasonal sampling from 4 stations. The number of taxa identified in the present study was comparatively higher than the taxa identified in previous studies that were performed in the southern Black Sea (Satilmis et al., 2003, 2014; Şahin & Düzgüneş 2019). Of the 33 fish species collected at larval or egg stages, many were identified at early life stages in earlier studies in the Black Sea (Selifonova, 2012; Satilmis et al., 2014; Mavruk & Örek, 2017; Klimova and Podrezova, 2018; Şahin & Düzgüneş, 2019; Klimova et al., 2021). Three species, however, *Neogobius melanostomus* (Gobiidae), *Mesogobius batrachocephalus* (Gobiidae), and *Parablennius incognitus* (Blennidae) that were identified in the present study were not represented in those surveys. Gobiidae and Blennidae are among the richest families represented with 33 and 10 species in the Black Sea, respectively (Yankova et al., 2014; Prazdnikov, 2023). Yet, both of these families are underestimated in ichthyoplankton surveys possibly for being hard to morphologically distinguish from one another due to the fact that distinctive morphological characters are not phylogenetically informative (Mavruk et al., 2022).

Sequences of sole specimens were assigned to *Pegusa impar* with high similarities (>99%). While the distribution range of *P. impar,* illustrated in the FishBase global biodiversity information system, does not cover the Black Sea, a recent checklist (Karataş et al., 2021) suggests *P. impar* distribution in the Black Sea. The generated *P. impar* DNA barcode sequence is the first sequence from the Black Sea population of the species. To be accurate, more specimens are required to clarify this discrepancy. To avoid further error, the specimens were identified at the genus level as *Pegusa* sp. Similarly, barcode sequences of one of the blennid specimens were assigned to *Salaria basillisca* with high similarity (>99.3%) followed by 99.03% similarity with *S. pavo*. *S. basillisca* are native to the Mediterranean Sea and their distribution range does not cover the Black Sea (Karataş et al., 2021). In the literature, there is no available barcode sequence from the Black Sea which might be the main reason of the misidentification. More specimens are required to clarify the discrepancy. To avoid further error, the specimens were identified at the genus level as *Salaria* sp.

Overall, presence of 189 fish species was reported from the Black Sea (Yankova et al., 2014) 154 of which were listed in the fish checklist for the Black Sea coast of Turkey (Bilecenoğlu et al., 2014) and most of these fish species are known to produce planktonic eggs or larvae. According to Mavruk and Örek (2017), a total of 73 fish species were identified during the ichthyoplanktonic stage in previous studies. Although our survey was localized, presence of 33 species was genetically identified from seasonal sampling from 4 stations without replication. The accuracy of fish biodiversity detection could be improved by increasing monitoring duration, replication, area of coverage, and hauling time.

Comparatively higher density of eggs than larvae reported here is similar to published studies in the Black Sea (Satilmis et al., 2014; Klimova & Podrezova, 2018; Şahin & Düzgüneş, 2019). The dominance of *E. encrasicolus* eggs was not surprising since this species is distributed abundantly in the Black Sea. In previous studies, *E. encrasicolus* has been reported as the most dominant group in the ichthyoplankton. In fact, Şahin and Düzgüneş (2019) found that *E. encrasicolus* comprised 89.8% of their egg catch and 47.4% of their larva catch. Klimova and Podrezova (2018) found that *E. encrasicolus* comprised 93.5% of their total ichthyoplankton catch in July, a remarkable similarity to the 91.5% reported in the present study.

The species presented in this study with comparatively lower abundance could be underestimated due to the benthic orientation of demersal species. Moreover, it is worth mentioning that ichthyoplankton collections were performed during the daylight hours. Dial changes in ichthyoplankton assemblages have demonstrated that densities of larvae collected at night hours exceeded those during daylight due to vertical migration (Bowles et al., 1978; Lima et al., 2016) and/or gear avoidance (Röpke, 1989; Flores-Coto et al., 2000). Moreover, due to the sampling once in three months (once in each season) and considering the seasonality of fish spawning, some species may have been overlooked or underestimated. Some fish species have a short spawning seasons. Therefore, with the limited sampling, their spawn may have been missed. It is also worth mentioning that, classifying seasonality by calendar month and not by actual water temperature also result in variability in the observed seasonality. Sampling day and night for a full year with short intervals would ideally increase the number of encountered fish species and would better reflect the community structure.

The results of the present study indicate that seasonality was a stronger influencer of the ichthyoplankton assemblages. Ichthyoplankton community composition and abundance are influenced by fish spawning behaviors and environmental processes. Most of the fish species in the Mediterranean Sea are reported as summer spawners (Tsikliras et al., 2010) which indicates that seasonality is important for fish spawning and assemblages. The influence of seasonality in ichthyoplankton assemblages was also reported from different parts of the world as well as from the Black Sea (Satilmis et al., 2014; Lima et al., 2016). Temperature is the main environmental driver

that shapes seasonal community composition. As an important environmental factor affecting fish physiology, it also affects fish reproductive activity (Hou et al., 2021).

Conclusions

In conclusion, the highest density of fish egg and larvae were found in summer season and species richness and abundance were higher in coastal stations, yet no distinct difference was observed between sampling station in terms of community composition. Seasonal ichthyoplankton communities are shaped by environmental factors and temperature is the main driver. The present study, to the best of our knowledge, is the first ichthyoplankton study in the Black Sea that used the DNA barcoding technique for species identification. DNA barcoding was an efficient method for identifying the fish species in the Black Sea during their early life stages. A total of 33 taxa were identified with limited sampling effort. As species identification through DNA barcoding requires a comprehensive database and due to the lack of available reference sequences from the Black Sea, 2 out of 33 taxa were identified at the genus level. The creation of a comprehensive DNA barcode database for the fish species of the Black Sea would allow accurate identification of ichthyoplankton specimens rapidly.

Ethical Statement

All applicable institutional guidelines for the care and use of animals were followed by the author.

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Author Contribution

This study is performed by single author.

Conflict of Interest

The author declares no conflict of interest.

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Table S1. Environmental parameters

Station	Season	Temperature (°C)	Salinity (ppt)	O2 (mg/l)	Sigma-t (kg/m³)	Conductivity (µs/m)	Chl-a (µg/l)	NH3 (µmol/l)	NO2 (µmol/l)	NO3 (µmol/l)	SiO4 (µmol/l)	PO4 (µmol/l)	TOC (µmol/l)
K0	Autumn	19.91	18.07	8.26	11.92	26.34	1.45	0.077	0.002	0.811	1.206	0.345	6.01
K1	Autumn	19.71	18.02	11.75	11.93	26.16	3.61	0.355	0.015	1.698	1.116	0.201	7.24
K2	Autumn	19.64	18.07	10.22	11.98	26.18	1.28	0.073	0.016	1.01	0.956	0.134	2.97
K3	Autumn	19.60	17.99	11.11	11.93	26.06	1.54	0.033	0.043	0.738	0.218	0.322	6.18
K0	Winter	9.58	18.29	10.17	10.99	20.91	1.622	0.03	0.022	1.858	0.536	0.121	29.92
K1	Winter	9.97	18.26	9.61	13.92	21.09	0.229	0.085	0.035	1.435	0.908	0.124	10.19
K2	Winter	9.94	18.09	15.21	13.79	20.89	3.699	0.078	0.052	1.942	0.793	0.137	118.25
K3	Winter	9.90	18.24	9.67	13.92	21.04	4.837	0.081	0.069	1.721	0.793	0.14	6.18
K0	Spring	13.59	18.22	10.17	13.34	23.01	0.384	0.018	0.02	0.401	0.417	0.026	17.6
K1	Spring	13.84	18.05	11.38	12.33	22.61	1.401	0.097	0.045	0.349	0.842	0.047	44
K2	Spring	14.71	17.96	13.53	12.93	23.31	0.359	0.18	0.019	0.445	0.901	0.022	27.97
K3	Spring	12.23	18.01	14.11	13.40	22.03	0.478	0.089	0.035	0.323	0.608	0.066	22.58
K0	Summer	25.86	17.16	7.73	13.17	29.91	0.498	0.056	0.022	0.346	0.371	0.045	234.3
K1	Summer	26.36	17.99	10.21	10.15	29.94	0.131	0.106	0.039	0.116	0.192	0.032	16.6
K2	Summer	25.63	18.14	7.22	10.47	29.73	0.797	0.116	0.038	0.057	0.189	0.039	58.96
K3	Summer	26.13	17.78	7.73	10.06	29.49	1.131	0.063	0.034	0.123	0.047	0.019	23.73