Temporal Variations in Abundance and Species Richness of Zooplankton with Emphasis on Ichthyoplankton in the Subtidal Waters of Umm Al-Namil Island, Northwestern Arabian Gulf of the ROPME Sea Area

Mohammad Ali, Matrah Al-Mutairi, M.N.V. Subrahmanyam, Sasini Isath, Mohammad A. AlAwadi, P. Niranjan Kumar, Khaled Al-Hebini and Samira A.S. Omar

Kuwait Institute for Scientific Research, P.O. Box: 24885, Safat-13109, State of Kuwait
Regional Organization for the Protection of the Marine Environment (ROPME), P.O. Box: 26388, Safat-13124, State of Kuwait

Abstract: Zooplankton, including the ichthyoplankton, abundance and species richness over time in the subtidal waters of Umm Al-Namil Island, Kuwait Bay, were sampled and quantified from September 2016 to August 2017. At the same period, physicochemical measurements (i.e., water temperature, pH, salinity, dissolved oxygen and nutrient concentrations) occurred. A total of 9 larval fish families were identified: Acropomatidae, Bregmacerotidae, Bythitidae, Clupeidae, Engraulidae, Leiogantidae, Platycephalidae, Pseudochromidae and Sparidae, in addition to fish eggs. Other zooplankton were mainly represented by Copepoda, followed by Radiolaria and Molluska larvae. Generally, some sampling events (i.e., months) had 100% fish larvae, while others had 0% fish larvae. The physicochemical parameters showed variations at each sampling event as well as within the same season. Total zooplankton (including ichthyoplankton) mean abundance was highest in summer (22.65 ± 2.85 ind.5l⁻¹), while winter (18.13 ± 1.64 ind.5l⁻¹) and autumn (17 ± 2 ind.5l⁻¹) mean abundance values did not significantly vary. The lowest mean abundance was observed in spring (14.33 ± 1.67 ind.5l⁻¹). Mean species richness was highest during spring (7.22 ± 1.66), but not significantly different from autumn (7 ± 2). No significant difference was observed between winter (6.73 ± 1.64) and summer (5.90 ± 2.85). Overall, the results indicate that zooplankton species richness and abundance in Umm Al-Namil Island varied temporally in response to fluctuations in environmental conditions. Primary among these fluctuations is water temperature at different seasons of the year.

Keywords: Fish Larvae and Eggs, Zooplankton Species Richness, Tidal Zones, Kuwait Bay

Introduction

Zooplankton and ichthyoplankton are fundamentally different fractions of pelagic communities. Zooplankton individuals spend their entire life cycle as plankton: Larval fishes are the temporary meroplanktonic stages of individuals that are for the most part nektonic; ichthyoplankton abundances reflect spawning locales and suitability of conditions for larval survival and recruitment to adult populations. Conditions affecting zooplankton and ichthyoplankton distribution and abundance may be quite different. Most studies on larval fish have been oriented toward recognizing and identifying the scales of the passive and active mechanisms, which determine distributional patterns and the results have been diverse and heavily biased toward the spatio-temporal scale, on which the study was designed (Kingsford and Choat, 1989; Castello et al.).
1991; Castro et al., 2000) a monthly (or more frequent) sample scheme could reveal seasonal signals in abundance and distribution of larval fish related to biological mechanisms (Moser and Pommeranz, 1999).

Beckley and van der Lingen (1999) described seasonal and spatial relationships between larval fish abundance and environmental conditions principally with temperature. Similarly, Smith et al. (1999), as well as Gray and Miskiewicz (2000) found seasonal changes in the composition and structure of larval fish species in southeast Australian waters with respect to regional oceanography. Seasonal larval fish abundance has also been coupled with oceanographic features such as areas strongly influenced by upwelling events, including Chile (Loeb and Rojas, 1987; Balbontin and Bravo, 1999; Castro et al., 2000), the California Current (McGowen, 1993) and the Benguela Current (Olivar and Shelton, 1993). Seasonality in larval fish abundance is also reflected by the composition of the species assemblages, in which, depending on the time of the year, it is possible to identify diverse groupings, which may not necessarily represent similarities in adult habitat (McGowen, 1993). Moreover, these patterns could also be linked to local, regional or global productivity (Hill et al., 1998) and also show important inter-annual variability (Bakun, 1996).

Despite the existence of reproductive seasonality, larval fish species may also show differences in their spatial patterns of abundance (e.g., with respect to bathymetry or distance to shore). These patterns are particularly strong in areas very near to the coast (Kingsford and Choat, 1989), or in areas with ample tidal regimes such as estuaries (Kingsford and Suthers, 1996). Short-term coupling between physical processes and biological mechanisms can strongly modify the distribution and abundance of larval fish (Harris and Cyrus, 2000). There is also evidence for a positive association between the occurrence of larval fish and other biological entities, e.g., jellyfish (Kingsford, 1993) and chaetognaths (Baier and Purcell, 1997). In this sense, the location and abundance of larval stages may in some cases exhibit a strong relationship with the type of habitat or spawning grounds of the adult segments of those populations (Hernández-Miranda et al., 2003).

This study was performed during the low tide at the start of the subtidal zone (the area at the end of the intertidal zone where subtidal waters start) up to 10 cm depth off Umm Al-Namil Island, as it forms an important transition area between intertidal and subtidal zones. The aims of this study are to determine if, (1) seasonality in larval fish abundance and species richness at the start of the subtidal zone with notes on other zooplankton and (2) short-term coupling between fish larvae and physicochemical features, with notes on other zooplankton, using the integration of biological data (i.e., larval abundance and species richness) and physicochemical data (e.g., temperature, salinity, pH and dissolved oxygen concentration) at the start of the subtidal zone.

**Materials and Methods**

**Study Site**

Umm Al-Namil Island (29°23′14.3″N 47°52′16.3″E) is one of the smallest islands among the nine islands present within Kuwait’s territorial waters in the northwestern part of the Arabian (= Persian) Gulf of the Regional Organization for the Protection of the Marine Environment (ROPME) Sea Area. ROPME Sea Area is divided into three areas: (i) inner, (ii) middle and (iii) outer sea areas. The study area herein lies within the inner ROPME Sea Area. The island is around 600 m away from mainland of Kuwait. It is situated at the southwestern corner of Kuwait Bay along the northwestern side of Sulaibikhat Bay (Fig. 1). It is oriented in a northeast-southwest direction and has a drumstick shape (Al-Zamel et al., 2007). The island narrows to 75 m in the southwestern side and is approximately 800 m long by 300 m wide at the eastern side. Along the eastern side of the main tidal channel of the island, maximum water depth is about 4.5 m (Al-Zamel et al., 2007). The island is bounded by well-developed tidal flats. Supratidal flats consist of gypsum and anhydrite, which form most of the coastal dunes in the area and the intertidal flat is hard and accompanied by oyster mounds, whereas the subtidal flat is composed of soft muddy sand with high productivity (Al-Zamel et al., 2007).

**Sampling and Analysis**

Zooplankton samples were collected during the period 2016-2017 in monthly intervals covering 4 seasons representing autumn (October and November), winter (December, January and February), spring (March and April) and summer (May, June, July, August and September). Sampling was performed during low tide at the start of the subtidal zone (the area at the end of the intertidal zone where subtidal waters start) at depth up to 10 cm off Umm Al-Namil Island. A unique number has been assigned for each sampling month; called event (Table 1). The sample of event 11 (July, 2017) was accidentally lost before analysis, thus not represented between events 10 and 12 (Table 1).

Samples were collected using a one-liter sampling bottle and poured into a 110-microns mesh for five times. Samples were fixed in 75% ethanol-seawater solution. Ichthyoplankton and other zooplankton were sorted from the entire sample using Olympus SZ40 and Carl Zeiss® Stemi DV4 stereomicroscopes. The fish larvae were identified up to the family level through their morphological characteristics, while other zooplankton were identified to the lowest taxon possible guided with local identification books.
(Richards, 2008; Al-Yamani and Pursova, 2003; Al-Yamani et al., 2011a; 2011b) and other international references (Lipson and Moran, 1974; Moser et al., 1983; Leis and Trnski, 1989; Neira et al., 1998; Richards, 2001). The term “unidentified/distorted larvae” was used for the larvae that were damaged and hard to distinguish their characteristics. The abundance of each plankton was calculated as individual in 5 liters (ind. 5l-1) since 5 liters of water were collected and filtered through a 110-microns mesh for the collection of all zooplankton in this study. All individuals in the collected samples were counted and identified.

Table 1: The sampled months during the study period in the subtidal area of Umm Al-Namil Island. The “Event” column refers to sampling event. Each event has assigned a unique number that refers to the month and year where sampling occurred. This was done in order to make figure representation easier to read. The table also shows seasons related to each month. The sample of event 11 (July, 2017) was accidentally lost before analysis and the month have already passed and that is why event 11 is not presented between events 10 and 12.

| Month, Year  | Season | Event |
|--------------|--------|-------|
| September, 2016 | Summer | 1     |
| October, 2016   | Autumn | 2     |
| November, 2016  | Autumn | 3     |
| December, 2016  | Winter | 4     |
| January, 2017   | Winter | 5     |
| February, 2017  | Winter | 6     |
| March, 2017     | Spring | 7     |
| April, 2017     | Spring | 8     |
| May, 2017       | Summer | 9     |
| June, 2017      | Summer | 10    |
| August, 2017    | Summer | 12    |

Fig. 1: Map of Kuwait showing the location of Umm Al-Namil Island in Kuwait Bay, northwestern Arabian (= Persian) Gulf, along with the sampling location represented by a red triangle at the start of the subtidal zone (29°22′43.4496″N 47°52′4.3176″E) during low tide.
Physicochemical and Biological Parameters

The physicochemical parameters showed variations at each sampling event as well as within the same season (Figs. 2 and 3). Dissolved oxygen concentration ranged between 5 to 8.2 mg.l⁻¹ at events 5 and 12 in winter and summer, respectively (Fig. 2A). Seawater temperature ranged between 7.2 to 29.4°C at events 4 and 1 in winter and summer, respectively (Fig. 2B). Temperature generally showed a consistent pattern of variation with season (Figs. 2B and 3A). Salinity ranged between 42 to 47.1 psu at events 3 and 12 in autumn and summer, respectively (Fig. 2C). The pH values ranged between 5.2 to 10 at events 4 and 8 in winter and spring, respectively (Fig. 2D). The level of pH was highest in spring compared to other seasons (Figs. 2D and 3D). Nitrate level ranged from 0.02 (events 6 in winter and 7 in spring) to 0.8 mg.kg⁻¹ at event 4 in winter (Fig. 2E). The level of nitrates was almost the same throughout events 2, 6-12 (Fig. 2E) without significant variations unlike events 1, 3, 4-5 (Fig. 2E). Phosphates showed almost a consistent pattern with events but the lowest value was observed in summer at events 9 and 10 being 0.19 mg.kg⁻¹ and highest at event 6 in winter being 0.7 mg.kg⁻¹ (Fig. 2F). Generally, the level of phosphates did not vary significantly among autumn, summer and spring (Figs. 2F and 3H) but was highest in winter being 0.5 ± 0.058 mg.kg⁻¹ (Figs. 2F and 3H). Silicates did not show a consistent pattern with events but it was highest at event 12 in summer (2.71 mg.kg⁻¹) and lowest at event 6 in winter (0.61 mg.kg⁻¹) (Fig. 2G). Nitrites were lowest in autumn at events 2 and 3 with the same value (0.02 mg.kg⁻¹), while the highest value (0.58 mg.kg⁻¹) was recorded at event 4 in winter (Fig. 2H). Zooplankton abundance (ind.5l⁻¹) was highest at event 12 in summer (39 ind.5l⁻¹) and lowest at event 8 in spring (1 ind.5l⁻¹) (Fig. 2I). Mean abundance was highest in summer (22.65 ± 2.85 ind.5l⁻¹), while winter (18.13 ± 1.64 ind.5l⁻¹) and autumn (17 ± 2 ind.5l⁻¹) did not significantly vary (Fig. 3I) and the lowest mean abundance was observed during spring (14.33 ± 1.67 ind.5l⁻¹) (Fig. 3I). However, abundance did not show a clear pattern with events (Fig. 1I). Species richness was observed highest at events 2 (autumn), 6 (winter), 7 (spring) and 9 (summer) being all 8 species (Table 5 and Fig. 2J) while the lowest was observed at events 4 (winter) and 8 (spring) being all 1 species (Table 5 and Fig. 2J). Species richness did not significantly vary with season (Fig. 3J).

Species Richness-Abundance Relationship and the Effect of Season, Temperature, Salinity and Dissolved Oxygen Concentration

Kruskal-Wallis test showed that the effect of temperature on zooplankton abundance was significant (Chi-Square = 53, df = 10, p = 7.446 × 10⁻⁸) as well as the effect of salinity (Chi-Square = 53, df = 10, p = 7.446 × 10⁻⁸) and the effect of dissolved oxygen (Chi-Square= 39.28, df = 8, p = 4.368 × 10⁻⁶) (Table 2). Season had no effect on abundance (Chi-Square = 1.56, df = 3, p = 0.67) (Table 2). The same statistical test also showed no significant effect of season on species richness (Chi-Square = 1.56, df = 3, p = 0.67), while the effect of temperature on the species richness was significant (Chi-Square = 53, df = 10, p = 7.446 × 10⁻⁸) (Table 3). The effect of salinity on species richness was also significant (Chi-Square = 53, df = 10, p = 7.446 × 10⁻⁸), as well as the effect of dissolved oxygen on species richness (Chi-Square = 39.28, df = 8, p = 4.368 × 10⁻⁶) (Table 3). These statistical findings almost coordinate with the mean values of species richness and abundance at each season as shown in Fig. 2I and 2J, being not significantly different. The abundance showed to statistically significantly affect the species richness in this study irrespective of season having a direct relationship (Chi-Square = 53, df = 10, p = 7.446 × 10⁻⁸) (Table 4 and Fig. 4A). The opposite was not true and species richness had no effect on the abundance (Chi-Square = 1.27, df = 3, p = 0.74) (Table 4 and Fig. 4B).

Species Composition

Generally, ichthyoplankton were encountered during events 2 to 9 (Table 5) and fish eggs were only observed in autumn (events 2 and 3), while fish larvae were found during events 4 to 6 in winter as well as during spring at events 7 and 8 and in summer at events 9 and 10. The highest variation in larval fish was encountered during events 6 and 9 being five families (Table 5). Events 3, 4 and 8 consisted of 100% ichthyoplankton (Table 5 and Fig. 5). Whereas event 4 sample encompassed only
Sparidae larvae (Table 5 and Fig. 5). Despite 1 fish egg observed at event 3, the rest of the sample was only Pseudochromidae larvae (Table 5 and Fig. 5). Despite the lowest abundance (1 ind.5l⁻¹) and lowest species richness (1) at event 8 compared to other events, the sample at that event had only a single Clupeidae larva (Table 5).

The percentage of ichthyoplankton among the other zooplankton collected at events 2, 5, 6, 7 and 9, were 65, 18.18, 77.27, 50 and 66.67%, respectively (Fig. 5). At event 2, ichthyoplankton were represented by Pseudochromidae larvae (9 ind.5l⁻¹) and fish eggs (4 ind.5l⁻¹) (Table 5). The rest of the sample was mainly composed of copepods [harpacticoids (4 ind.5l⁻¹), Corycaeus sp. (1 ind.5l⁻¹) and nauplii (1 ind.5l⁻¹)] followed by Polychaeta larvae (1 ind. 5l⁻¹) (Table 5). At event 5, ichthyoplankton were represented by Sparidae (1 ind.5l⁻¹) and Engraulidae (1 ind. 5l⁻¹) (Table 5). The rest of the sample was mainly composed of cyclopoid copepods (4 ind.5l⁻¹), ostracods (3 ind. 5l⁻¹), and harpacticoid copepods (4 ind. 5l⁻¹) (Table 5). At event 6, ichthyoplankton were represented by Engraulidae larvae (2 ind.5l⁻¹), Platynereisidae larvae (1 ind.5l⁻¹), Acropomatidae larvae (6 ind.5l⁻¹), Bythitidae larvae (6 ind.5l⁻¹) and unidentified/distorted larva (2 ind. 5l⁻¹) (Table 5). The rest of the sample was mainly composed of cyclopoid copepods (3 ind.5l⁻¹), followed by both harpacticoid copepods (1 ind.5l⁻¹) and copepod nauplii (1 ind.5l⁻¹) (Table 5). At event 7, ichthyoplankton were represented by Sparidae larvae (6 ind.5l⁻¹), Bremgacerotidae larvae (1 ind.5l⁻¹), and unidentified/distorted larva (1 ind.5l⁻¹) (Table 5). The rest of the sample was divided between copepods [Acartia ohtsuki (2 ind. 5l⁻¹), Pseudodiaptomus arcticus (1 ind.5l⁻¹) and Temora turbinata (1 ind.5l⁻¹)] and Molluska larvae [Gastropoda larvae (2 ind.5l⁻¹), Bivalvia larvae (2 ind.5l⁻¹)] (Table 5). At event 9, ichthyoplankton were represented by Sparidae larvae (1 ind.5l⁻¹), Bythitidae larvae (2 ind.5l⁻¹), Platyecephalidae larvae (3 ind.5l⁻¹), Leioognathidae larvae (1 ind.5l⁻¹), and Acropomatidae larvae (3 ind.5l⁻¹) (Table 5). The rest of the sample was composed of copepods [cyclopoids, harpacticoids and nauplii of 1 ind.5l⁻¹] each (Table 5).

Events 1, 10 and 12, had no ichthyoplankton in their samples (Table 5, Fig. 5). Event 1 was dominated by radiolarians (20 ind.5l⁻¹), followed by bivalve larvae (7 ind.5l⁻¹) and ostracods (3 ind.5l⁻¹), while the rest of the zooplankton species/groups had lower abundance [2 ind. 5l⁻¹ of juvenile Acartia sp. and 1 ind.5l⁻¹ of each copepod nauplii and Gastropoda larvae] (Table 5). Event 10 was dominated by Oikopleura dioica (2 ind.5l⁻¹), while the rest were Chaetognatha and a cyclopoid (probably parasitic Bomofoidea) copepod (each 1 ind. 5l⁻¹) (Table 5). Like event 1, event 12 was dominated by radiolarians (20 ind.5l⁻¹), followed by bivalve larvae (15 ind.5l⁻¹) and Gastropoda larvae (4 ind.5l⁻¹) (Table 5). Furthermore, it has been observed that the samples of events 1 and 12 contained so much silica.

Fig. 2: Physicochemical and biological parameters at each sampling event (Event) as explained in Table 1 in the subtidal waters of Umm Al-Namil Island. (A) Dissolved Oxygen Concentration (mg.l⁻¹), (B) Seawater Temperature (°C), (C) Salinity (PSU), (D) pH, (E) Nitrates (mg.kg⁻¹), (F) Phosphates (mg.kg⁻¹), (G) Silicates (mg.kg⁻¹), (H) Nitrites (mg.kg⁻¹), (I) Zooplankton Abundance (individual. 5l⁻¹) and (J) Species Richness (S). Colors represent seasons as follows: White for winter, black for autumn, light grey for spring and dark grey for summer. The sample of event 11 (July, 2017) was accidentally lost before analysis and the month have already passed and that is why event 11 is not presented between events 10 and 12.
Fig. 3: Mean values with standard error bars for physicochemical and biological parameters at each season in the subtidal waters of Umm Al-Namil Island. (A) Mean Seawater Temperature (°C), (B) Mean Salinity (PSU), (C) Mean Dissolved Oxygen Concentration (mg.l⁻¹), (D) Mean pH, (E) Mean Silicates (mg.kg⁻¹), (F) Mean Nitrates (mg.kg⁻¹), (G) Mean Nitrites (mg.kg⁻¹), (H) Mean Phosphates (mg.kg⁻¹), (I) Mean Zooplankton Abundance (individual. 5l⁻¹) and (J) Mean Species Richness (S). Absence of some error bars is due to the very small variation.

Fig. 4: Plot A shows a direct relation between species richness and abundance while the opposite is not true as shown in plot B. Colors represent seasons as follows: Antique white for autumn, black for spring, grey for summer and dark grey for winter. Such clear relationship in plot A appeared in all seasons. Table 4 explains the relationship in statistical terms.
Fig. 5: Percentage of ichthyoplankton (%) among the collected zooplankton samples at each sampling event. The maximum percentage was 100% at events 3, 4 and 8, while no ichthyoplankton were observed at events 1, 10 and 12. Event 11 sample was lost as explained in the Material and Methods section including Table 1 caption. Each color represents a season as shown on the legend.

Table 2: Kruskal-Wallis test showing the effect of season, seawater temperature, salinity and dissolved oxygen concentration on zooplankton abundance. The effect of season on abundance was not significant (Chi-Square = 1.56, df = 3, \( p = 0.67 \)), while the effect of temperature on the abundance was significant (Chi-Square = 53, df = 10, \( p = 7.446 \times 10^{-08} \)). The effect of salinity on abundance was also significant (Chi-Square = 53, df = 10, \( p = 7.446 \times 10^{-08} \)), as well as the effect of dissolved oxygen on abundance (Chi-Square = 39.28, df = 8, \( p = 4.368 \times 10^{-06} \)). Significance codes of p values: S = significant, N = not significant.

| Explanatory variable | Chi-square | DF | \( p \)   |
|----------------------|------------|----|-----------|
| Season               | 1.56       | 3  | 0.67N     |
| Temperature          | 53.00      | 10 | 7.446\times10^{-08} |
| Salinity             | 53.00      | 10 | 7.446\times10^{-08} |
| Dissolved Oxygen     | 39.28      | 8  | 4.368\times10^{-06} |

Table 3: Kruskal-Wallis test showing the effect of season, seawater temperature, salinity and dissolved oxygen concentration on zooplankton species richness. The effect of season on species richness was not significant (Chi-Square = 1.56, df = 3, \( p = 0.67 \)), while the effect of temperature on the species richness was significant (Chi-Square = 53, df = 10, \( p = 7.446 \times 10^{-08} \)). The effect of salinity on species richness was also significant (Chi-Square = 53, df = 10, \( p = 7.446 \times 10^{-08} \)), as well as the effect of dissolved oxygen on species richness (Chi-Square = 39.28, df = 8, \( p = 4.368 \times 10^{-06} \)). Significance codes of p values: S = significant, N = not significant.

| Explanatory variable | Chi-Square | DF | \( p \)   |
|----------------------|------------|----|-----------|
| Season               | 6.13       | 3  | 0.105N    |
| Temperature          | 53.00      | 10 | 7.446\times10^{-08} |
| Salinity             | 53.00      | 10 | 7.446\times10^{-08} |
| Dissolved Oxygen     | 44.28      | 8  | 5.029\times10^{-07} |

Table 4: Kruskal-Wallis test showing the effect of abundance on species richness and vice versa. The effect of abundance on species richness was significant (Chi-Square = 53, df = 10, \( p = 7.446 \times 10^{-08} \)), while the effect of species richness on abundance was not significant (Chi-Square = 1.27, df = 3, \( p = 0.74 \)). Significance codes of p values: S = significant, N = not significant.

| Explanatory variable | Chi-square | DF | \( p \)   |
|----------------------|------------|----|-----------|
| Abundance            | 53.00      | 10 | 7.446\times10^{-08} |
| Species Richness     | 1.27       | 3  | 0.74N     |
Table 5: List of ichthyoplankton and zooplankton found at each sampling event (i.e., month) with season in this study in addition to total abundance, species richness and individual abundance. The abundance is estimated as individual per 5 liters (ind.5l⁻¹). The table also shows the season that coincided with every month.

| Month. Year | Event | Season | Species                          | Individual species abundance |
|------------|-------|--------|----------------------------------|------------------------------|
| Sept. 2016 | 1     | Summer | Bivalvia larvae                  | 7                            |
|            |       |        | Juvenile Acartia sp.             | 2                            |
|            |       |        | Coepod naupili                   | 1                            |
|            |       |        | Ostracoda                        | 3                            |
|            |       |        | Gastropoda larvae                | 1                            |
|            |       |        | Radiolaria                       | 20                           |
| Oct. 2016  | 2     | Autumn | Coepod naupili                   | 1                            |
|            |       |        | Corycaeus sp.                    | 1                            |
|            |       |        | Unknown harpacticoid             | 1                            |
|            |       |        | Euterpina acutifrons             | 2                            |
|            |       |        | Clytemnestra scutellate          | 1                            |
|            |       |        | Polychaeta larvae                | 1                            |
|            |       |        | Fish eggs                        | 4                            |
|            |       |        | Pseudochromidae larvae           | 9                            |
| Nov. 2016  | 3     | Autumn | Fish eggs                        | 1                            |
|            |       |        | Pseudochromidae larvae           | 4                            |
| Dec. 2016  | 4     | Winter | Sparidae larvae                  | 30                           |
| Jan. 2017  | 5     | Winter | Sparidae larvae                  | 1                            |
|            |       |        | Engraulidae larvae               | 1                            |
|            |       |        | Ostracoda                        | 3                            |
|            |       |        | Unknown harpacticoid             | 1                            |
|            |       |        | Unknown cyclopoid                | 4                            |
|            |       |        | Euterpina acutifrons             | 1                            |
| Feb. 2017  | 6     | Winter | Coepod naupili                   | 1                            |
|            |       |        | Unknown harpacticoid             | 1                            |
|            |       |        | Engraulidae larvae               | 3                            |
|            |       |        | Ostracoda                        | 2                            |
|            |       |        | Platycalanidae larvae            | 1                            |
|            |       |        | Acroplaxidae larvae              | 6                            |
|            |       |        | Bythitidae larvae                | 6                            |
|            |       |        | Unidentified/Distorted larvae    | 2                            |
| Mar. 2017  | 7     | Spring | Bivalvia larvae                  | 2                            |
|            |       |        | Gastropoda larva                 | 2                            |
|            |       |        | Acartia ohtsuki                  | 1                            |
|            |       |        | Pseudodiaptomus arabicus         | 2                            |
|            |       |        | Temora turbinata                 | 1                            |
|            |       |        | Sparidae larvae                  | 1                            |
|            |       |        | Bregmacoritidae larvae           | 1                            |
|            |       |        | Unidentified/Distorted larvae    | 1                            |
| Apr. 2017  | 8     | Spring | Clupeidae larvae                 | 1                            |
| May. 2017  | 9     | Summer | Coepod naupili                   | 1                            |
|            |       |        | Unknown harpacticoid             | 1                            |
|            |       |        | Unknown cyclopoid                | 1                            |
|            |       |        | Sparidae larvae                  | 1                            |
|            |       |        | Bythitidae larvae                | 5                            |
|            |       |        | Platycalanidae larvae            | 2                            |
|            |       |        | Leioantheridae larvae            | 3                            |
|            |       |        | Acroplaxidae larvae              | 1                            |
| Jun. 2017  | 10    | Summer | Oikopleura dioica                | 2                            |
|            |       |        | Chaetognatha                     | 1                            |
|            |       |        | Bomolochidae copepod (?)         | 1                            |
| Aug. 2017  | 12    | Summer | Bivalvia larvae                  | 15                           |
|            |       |        | Gastropoda larvae                | 4                            |
|            |       |        | Radiolaria                       | 20                           |

| Month. Year | Event | Season | Species                          | Individual species abundance |
|------------|-------|--------|----------------------------------|------------------------------|
|            |       |        | Species Richness                | 6                            |
|            |       |        | Total Abundance                  | 34                           |
|            |       |        | Species Richness                | 8                            |
|            |       |        | Total Abundance                  | 20                           |
|            |       |        | Species Richness                | 2                            |
|            |       |        | Total Abundance                  | 5                            |
|            |       |        | Species Richness                | 6                            |
|            |       |        | Total Abundance                  | 11                           |
|            |       |        | Species Richness                | 8                            |
|            |       |        | Total Abundance                  | 22                           |
|            |       |        | Species Richness                | 1                            |
|            |       |        | Total Abundance                  | 30                           |
|            |       |        | Species Richness                | 1                            |
|            |       |        | Total Abundance                  | 11                           |
|            |       |        | Species Richness                | 16                           |
|            |       |        | Total Abundance                  | 39                           |
Discussion

The Effect of Environmental Parameters on Zooplankton and Ichthyoplankton Distribution, Abundance and Species Richness

The effect of temperature, salinity and dissolved oxygen on both abundance and species richness of zooplankton including ichthyoplankton was significant, while the effect of season on these traits was not significant (Table 2 and 3). Also, the effect of abundance on species richness was significant (Table 4). In addition, the percentage of ichthyoplankton varied between events among the zooplankton community from 0 up to 100% (Fig. 5). Previous studies have showed that coastal areas are commonly used as nursery and spawning grounds by a variety of species that are otherwise 'ecologically' different, whether they live in various habitats as adults, such as benthos and intertidal zone, or exhibit distinctive spawning strategies, such as pelagic, demersal or beach spawning (Ellertsen et al., 1981; Frank and Leggett, 1983; Doyle and Ryan, 1989; Doyle et al., 1993; McGowen, 1993).

Larval fish assemblages are affected by salinity and temperature (Houde et al., 1986; Ndour et al., 2018; Zhang et al., 2019), the parameters that influence water density (Romeo et al., 2018). In addition, larval fish identify unique water masses through different temperatures (Mann and Lazier, 1991). In estuaries, plumes are characterized by vertical and/or horizontal gradients in temperature or salinity, which may be exploited by some larvae (Forward, 1989; 1990; Zhang et al., 2019). The same is applied on zooplankton as well (Johnson and Allen, 2012; Varadharajan and Soundarapandian, 2013; Berraho et al., 2019). Seawater temperature in this study ranged between 7.2 to 29.4°C at events 4 and 1 in winter and summer, respectively (Fig. 2B). Temperature generally showed a consistent pattern of variation with season (Figs. 2B and 3A). Generally, highest abundance and highest species richness (Table 5, Fig. 2I and 2J) were observed at warmer seasons (summer and spring) and this could have accounted for the significant relationship on both abundance and species richness (Tables 2-4, Fig. 4A and 4B). The same explanation could be applied to salinity as usually high salinity is the result of extremely high evaporation (Privett, 1959), which exceeds combined freshwater and rainfall inputs by over a factor of ten (Sheppard, 1993). Favorable habitats, on one aspect, have been defined by their physicochemical characteristics, mainly suitable to salinity and temperature conditions (Laprise and Dodson, 1993) as well as circulation patterns that promote transport or retention to nursery grounds (Harden Jones, 1969; Sinclair, 1988; Freitas and Muelbert, 2004).

Salinity is the major problem of the coastal environment (Vijayakumar et al., 2000). However, in this study, this is not an obstacle, as salinity ranged between 42 to 47.1 psu at events 3 and 12 in autumn and summer, respectively (Fig. 2C). The lowest salinity events could be related to rain in winter plus sewage runoff all the time near the investigating area but surely the evaporation and dryness in summer were high. Temperature is a major factor that controls the abundance and species richness in zooplankton and ichthyoplankton (Houde et al., 1986; Esteves et al., 2000; Mouny and Dauvin, 2002; Tackx et al., 2004; Ndour et al., 2018; Berraho et al., 2019; Zhang et al., 2019). Dissolved oxygen was reported to be always higher at the subsurface zone, irrespective of the season and followed a pattern very similar to surface water temperatures (Hernández-Miranda et al., 2003). In the present study, the water depth is more or less like a subsurface layer depth (up to 10 cm depth), thus mixing with wind is occurring all the time despite its velocity and the dissolved oxygen concentration ranged between 5 (events 5 and 6) to 8.2 (events 4 and 12) mg.l⁻¹, respectively (Fig. 2A). It has been reported that locations of high zooplankton biomass correspond to the zones of high concentration of dissolved oxygen in Senegal and Guinea (Ndour et al., 2018).

Other factors affecting larval fish abundance and distribution are water turbidity, tidal cycles and spawning time, as well as food availability and feeding habits (Muhamad and Rahim, 2014; Souza and Junior, 2019; Zhang et al., 2019). This is because meteorological and oceanographic features are often associated with seasonal patterns of abundance of larval fish (Hernández-Miranda et al., 2003; Ndour et al., 2018; Souza and Junior, 2019; Zhang et al., 2019) and it can affect transportation and feeding of larvae by currents (Harden Jones, 1969; Sinclair, 1988; Freitas and Muelbert, 2004). In this study, food for fish larvae seems to be available from the zooplankton species encountered, particularly copepods and mollusk larvae (Table 5). Also, in another study at the same location on phytoplankton, they were highly abundant with high species richness, especially diatoms (Al-Mutairi et al., 2020). Furthermore, it has been reported that the most vulnerable (mainly consumed) groups in the Arabian Gulf food web are calanoid copepods, harpacticoid copepods, and diatoms (Ali, 2015). Therefore, this indicates that the planktonic food web is strongly supported by both primary producers and primary consumers. However, ichthyoplankton were absent at events 1, 10 and 12 and that could be attributed to the aforementioned factors by Muhamad and Rahim (2014), Souza and Junior (2019) and Zhang et al. (2019). In addition, this also depends on the type of adults present around the study area and their spawning season. For instance, seasonal changes of the abundance of larval Sardinella spp. were reported to be consistent.
with artisanal landings of sardines in the vicinity of Muscat, Oman (Al-Abri et al., 2017).

Furthermore, coastal environments such as fjords, bays and islands may form favorable habitats for the early-life stages of a huge number of fish living in various marine ecosystems (Frank and Leggett, 1983; Boehlert and Mundy, 1993; Leis, 1993; McGowen, 1993; Souza and Junior, 2019; Zhang et al., 2019). Favorable habitats, on one aspect, have been defined by their biological properties, mainly through the high abundance of food and low abundance of predators (Frank and Leggett, 1982; 1983; Leggett, 1985; Souza and Junior, 2019). In this study, the only planktonic predator observed was Chaetognatha (Baier and Purcell, 1997) at event 10 and only in extremely low abundance (1 ind. 5l\(^{-1}\)) (Table 5). As for larger predators, mainly planktivorous fish, this study was not concerned about fisheries but seems from the findings that they are not forming huge impact on ichthyoplankton as they were encountered at about 73% of the sampling events and at three (events 3, 4 and 8) of them they were the only plankton caught (Table 5). These findings could confirm what has been reported about low abundance of ichthyoplankton predators in such coastal areas (Frank and Leggett 1982; 1983) as well as food availability (Taggart and Leggett, 1987; Doyle and Ryan, 1989; Souza and Junior, 2019). As for water current and circulation, this study was not concerned about this aspect but coastal waters of bays and islands as in this study, generally do exhibit circulation patterns which enhance retention of the ichthyoplankton stages, as well as affecting the distribution of zooplankton in general (Harden Jones, 1969; Sinclair, 1988; Laprise and Peppin, 1995; Freitas and Muelbert, 2004; Muhamad and Rahim, 2014; Kodama et al., 2018).

The Effect of Environmental Parameters on Non-Ichthyoplankton Zooplankton Distribution, Abundance and Species Richness

Generally, copepods were present at 7 sampling events (1, 2, 5, 6, 7, 9 and 10), despite the level of abundance as well as species composition (Table 5). Such temporal variations in copepod diversity were also observed in Cintra Bay, Morocco (Berraho et al., 2019). However, temporal variations of the abundance of copepods were reported to be not significant in the southern Arabian Gulf and the Strait of Hormuz (Rezai et al., 2019). This could be due to the specific seasonal (Autumn and Summer) sampling as well as focusing on only neustonic zooplankton and not to mention that their stations are generally deep-water stations in Rezai et al. (2019) compared to this study. Another study in Bushehr (Northeastern Arabian Gulf) showed two peaks in zooplankton density observed in February and September (Mokhayer et al., 2017). In the study herein, the highest abundance values were observed at events 12 (August 2017) and 1 (September 2016) being 34 and 39 ind. 5l\(^{-1}\), respectively (Table 5). In winter and fall, events 4 (December 2016), 6 (February 2017) and 2 (October 2016), had the second highest abundance values being 30, 22 and 20 ind. 5l\(^{-1}\), respectively (Table 5). Despite the different aims and sampling areas of the study herein and Mokhayer et al. (2017), peaks of highest abundances occurred more or less at the same times.

Molluska larvae, either bivalves or gastropods, were only encountered at events 1, 7 and 12 (Table 5). Several bivalve mollusks were reported to spawn at certain times of the year, while others reported to spawn almost all-year-long with peaks at certain times in New Zealand (Booth, 1983). The spawning periods reported were relatively short being four months or less (Booth, 1983). This could also be applicable on planktonic bivalves encountered in this study but needs further and detailed examination, especially in relation to season and reproductive development around Umm Al-Namil Island and Kuwait coastal waters in general. As for gastropods, the spawning season for Bolinus brandaris (Family: Muricidae) was reported to be between May and July with a clear spawning peak from June to July in Ria Formosa Lagoon in Southern Portugal (Vasconcelos et al., 2012). In this study, Indothais lacera (Family: Muricidae) were observed with eggs on rocks in the intertidal zone of Umm Al-Namil Island on 27 May 2017 (event 9); suggesting spawning period, that could most probably be the start of it, however, this needs further and detailed examination, especially in relation to season and reproductive development for various gastropods to compare in details with the planktonic stages around Umm Al-Namil Island and Kuwait coastal waters in general. Nonetheless, the times (i.e., seasons) of encountering larval gastropods and bivalves in this study coincide more or less with the aforementioned previous reports.

Radiolaria was only observed at events 1 and 12, dominating the zooplankton abundance (Table 5). Their abundance in both events was 20 ind.5l\(^{-1}\) (Table 5). This could most probably be related to the highest level of silicates in these events compared to the rest (Fig. 2G). This could mostly be attributed to dust storms that commonly occur in the region including Kuwait (Safar, 1980; 1985; Anwar et al., 1986; Sabbah et al., 2018; Misak et al., 2019), since a single short duration of a dust deposition event could represent up to 30% of the total annual flux for silicates (Bergametti et al., 1989), in addition to the dissolution of shell material, particularly diatoms (Chester, 1990), that could have been washed (Frings et al., 2016) to the subtidal zone (i.e., sampling area) through water mixing. In addition, water discharge from Shatt Al-Arab River from Iraq introduces nutrients into the Arabian Gulf (Talling,
1980; Subba Rao and Al-Yamani 1998; Nezlin et al., 2007; Sheppard et al., 2010).

Only at event 5, the total abundance of copepods (6 ind.5l⁻¹) was higher than the other groups including ostracods (3 ind.5l⁻¹) and fish larvae (2 ind.5l⁻¹) (Table 5). At event 12, mollusk larvae (total = 19 ind.5l⁻¹) was the second most abundant group after radiolarian and no other zooplankton groups were observed at that time. In general, this shows that mollusks in the tidal zones are reproducing and their larvae are available for fish larvae as food along with benthic copepods (mainly harpacticoids as shown at events 2, 5, 6 and 9; Table 5). Also, our sampling method might have accounted for the differences in abundance as mentioned earlier.

Unlike the results of Grabe and Lees (1992; 1995), in this study, Decapoda larvae including penaeid and non-penaeid shrimp were not encountered. This could be due to current movement and predation by fish (Abdulrahiman et al., 2006; 2010; Kodama et al., 2018).

The Effect of Environmental Parameters on Ichthyoplankton Distribution, Abundance and Species Richness

Autumn, winter and spring, had witnessed the highest percentages of ichthyoplankton among the zooplankton sampled at events 3, 4 and 8, respectively and all being 100% (Fig. 5 and Table 5). The composition of ichthyoplankton at event 3 was fish eggs and larval Pseudochromidae, while the composition at event 4 was only Sparidae larvae and the composition at event 8 was only Clupeidae larvae (Table 5). Despite the level of abundance, Fish eggs were only encountered in autumn (events 2 and 3) as well as larval Pseudochromidae (Table 5). The percentage of ichthyoplankton at event 2 was 65% (Fig. 5). It might have been the spawning season of some adults of this family and that could be the reason why these larvae were only encountered at these times.

Clupeidae and Engraulidae larvae were reported to be estuarine residents, estuarine dependents, or marine visitors (Souza and Junior, 2019; Zhang et al., 2019). In Kuwait, these larvae were reported to be the most abundant among the larval fish community (Houde et al., 1986) as well as in Oman (Al-Abri et al., 2017) and west of Africa, particularly Senegal and Guinea (Ndour et al., 2018). In winter, eggs and larvae of clupeids were declined to low levels of abundance, while they were mostly abundant in spring-summer and early autumn in Kuwait waters (Houde et al., 1986). Such findings are similar to this study, in which larval clupeids were only encountered in spring (event 8) despite the level of abundance, which was 1 ind.5l⁻¹ with species richness value of 1 (Table 5) and that could be attributed the limitations of sampling as mentioned earlier. Furthermore, Houde et al. (1986) reported that the highest abundance of clupeids was in Kuwait Bay, including the areas near Umm Al-Namil Island. As for Engraulidae eggs and larvae, their abundance peaked in late spring-early summer, decreased in late summer and then reached a secondary peak in early autumn (Houde et al., 1986). In this study, larval engraulids were only encountered in winter at events 5 and 6 and have not been observed at any other season (Table 5). The abundance of Engraulidae larvae at events 5 and 6 was 1 and 2 ind.5l⁻¹, respectively (Table 5) and that could be attributed to the limitations of sampling as mentioned earlier. In this study, the mean temperature in winter was 10.053 ± 0.053°C and in autumn was 22.76 ± 0.053°C (mean ± se), while Houde et al. (1986) reported ~ 16 to 20°C in January and February 1980 [equivalent to events 5 and 6 in 2017 in this study, respectively (Fig. 2B, Table 1 and 5)]. Therefore, possible changes in the environment could have occurred.

The fish inhabiting the pools formed by tidal cycles on the rocky shore can be classified as either transients or residents, depending on the time period spent in this environment (Gibson, 1969; 1982; Grossman, 1982; Mahon and Mahon, 1994; Griffiths, 2003). Fish that spend all their life cycle in tidal pools are defined as resident and are generally small benthic fishes, such as the blennies and gobies; while the transient fish are defined as those that only spend part of their life in this environment, they are primarily infralittoral, but occur in tidal pools, specifically as juveniles (Gibson, 1982; Castellanos-Galindo et al., 2005). Sparidae [e.g., Diplodus argenteus (Barreiros et al., 2004)] were reported to be among the transient fish families (Barreiros et al., 2004; Dias, 2013). In this study, highest abundance of larval sparids was observed in winter at event 4 (30 ind.5l⁻¹) and at that event species richness was 1, i.e., 100% sparids (Fig. 5 and Table 5). The lowest abundance was 1 ind.5l⁻¹ observed at events 5 (winter) and 9 (early summer), while in spring (event 7) the abundance of these larvae was 6 ind.5l⁻¹ (Table 5). This could conform Houde et al. (1986) hypothesis that sparids have no specific spawning season as their abundance in Kuwait waters was relatively stable as also Grabe et al. (1992) had reported that these larvae were present throughout the year, however, this needs further investigation in terms of species spawning time. In addition, anglers were observed on the Island catching some sparids using hook and line, particularly Sparidentex hasta, which indicates that adult sparids do exist in these areas to feed, as their food is available, particularly mollusks (Abdulrahiman et al., 2010) and to spawn.

Platyccephalidae larvae are abundant in Kuwait waters, including Kuwait Bay where Umm Al-Namil Island is located (Houde et al., 1986). In Kuwait and the northern Arabian Gulf, cold winter might have ceased the spawning of platyccephalids (Houde et al., 1986). In this study, Platyccephalidae larvae were encountered at events 6 (late winter, temperature = 10.6°C) and 9 (early winter) in this study, Umm Al-Namil Island. As for Engraulidae eggs and larvae, their abundance peaked in late spring-early summer, decreased in late summer and then reached a secondary peak in early autumn (Houde et al., 1986). In this study, larval engraulids were only encountered in winter at events 5 and 6 and have not been observed at any other season (Table 5). The abundance of Engraulidae larvae at events 5 and 6 was 1 and 2 ind.5l⁻¹, respectively (Table 5) and that could be attributed to the limitations of sampling as mentioned earlier. In this study, the mean temperature in winter was 10.053 ± 0.053°C and in autumn was 22.76 ± 0.053°C (mean ± se), while Houde et al. (1986) reported ~ 16 to 20°C in January and February 1980 [equivalent to events 5 and 6 in 2017 in this study, respectively (Fig. 2B, Table 1 and 5)]. Therefore, possible changes in the environment could have occurred.

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summer, temperature = 20°C), with the abundance of 1 and 3 ind.51⁻¹, respectively (Table 5). This is more or less conforms with Houde et al. (1986) and Grabe et al. (1992) findings, as higher abundance was observed in warmer season over a colder season and in this case, it might not be a complete cessation of adult platycephalids spawning but lower fecundity in winter. The reproductive biology of Platycephalus indicus (Platycephalidae) in Kuwait waters was assessed through Gonadosomatic Index (GSI) and macroscopic assessment, demonstrated that spawning occurred from December to April, peaking in February (Ben Hasan et al., 2015). This is more or less conforms with the present findings, in which event 9 (May 2017) had the highest abundance of Platycephalidae larvae as mentioned earlier. As for event 6 (February 2017), the abundance was lower and that could be due to our sampling method or the spawning of another species of Platycephalidae family. However, for instance, the highest GSI values for both sexes of P. indicus were observed in January (late winter), while the lowest GSI values were observed in May (early summer) in the southeastern coast of the Arabian Gulf at Bandar Abbas in Iran near the Strait of Hormuz (Mohammadikia et al., 2014). Further investigation in terms of various adult platycephalids spawning periods is needed to compare with their planktonic stages in the vicinity of Umm Al-Namal Island and in Kuwait waters in general.

In Kuwait waters, larval Leiogonanthidae was reported to be absent or relatively rare in winter (Houde et al., 1986). In this study, such larvae were only encountered in summer (event 9) with the abundance of 1 ind.51⁻¹ (Table 5). This agrees more or less with Grabe et al. (1992), which reported that the highest abundance of larval leioganthids was during summer in Kuwait Bay. The spawning grounds of adult leioganthids reported to be at offshore over the deeper parts of Kuwait’s waters (Houde et al., 1986), though their larvae were found in coastal waters in this study. This indicates that further investigation is needed regarding the spawning grounds for each leioganthid species. Larval Bregmacerotidae were least abundant in winter in Kuwait’s waters (Houde et al., 1986; Grabe et al., 1992) and in this study they were only found in spring (event 7) (Table 5). Larval Bregmacerotidae were also reported to be most abundant in Kuwait Bay (Houde et al., 1986). In this study, due to the aforementioned collection method as well as possible changes in the environment, the larvae were rarely encountered. Furthermore, Bregmacerotidae and Bythitidae larvae were reported to from 0.47% and 0.24% of larval fish catch in Muscat, Oman, respectively (Al-Abri et al., 2017), while the percentage among larval fish in this study was 1.24% and 9.88%, respectively and that could also be attributed to the method of collection and small number of samples and the type of habitat sampled compared to the study of Al-Abri et al. (2017).

Nonetheless, the collections indicate that these larvae are not abundant in both studies.

Zooplankton and Ichthyoplankton Abundance and Species Richness Relationships

The effect of zooplankton abundance on zooplankton species richness was reported to be positively correlated in ballast water collected from transatlantic ships arriving at both Arctic and Great Lakes ports (Chan et al., 2014). Similar effect of zooplankton total abundance (including ichthyoplankton) on species richness was observed in this study (Table 4 and Fig. 4a). Though statistically significant (Table 4), it seems to be that species richness and total abundance had no clear relationship and that most probably due to our sampling method, relatively small number of samples collected, as well as the limited area of sampling since these organisms drift with water current and can be moved to different places (Harden Jones, 1969; Sinclair, 1988; Laprise and Peppin, 1995; Freitas and Muelbert, 2004; Muhama and Rahim, 2014; Kodama et al., 2018). These factors would also explain the non-significant relationship between species-richness and abundance (Table 4 and Fig. 4B). As mentioned earlier, in general, highest abundance and highest species richness were observed at warmer seasons (summer and spring) (Table 4, 5, Figs. 2I, 2J, 3I, 3J, 4A and 4B) and this could have accounted for the significant relationship on both abundance and species richness (Table 4, Fig. 4A and 4B). Such observations regarding higher abundance and species richness in zooplankton and ichthyoplankton in warmer seasons compared to colder seasons were previously reported in Kuwait (Houde et al., 1986; Michel et al., 1986a; 1986b; Al-Yamani and Pursova, 2003; Al-Yamani et al., 2004).

Conclusion

Fish larvae of various species and life stage require different environmental parameters at any given time (Sameoto, 1984). The results of this study showed that the end of the intertidal zone and the start of the subtidal zone is rich in various forms of zooplankton and ichthyoplankton. Therefore, a temporal monitoring is required in order to observe any changes in either physiochemical or biological factors with time (Muhammad and Rahim, 2014; Souza and Junior, 2019). Further elaborated studies are needed along with other subtidal waters of northwestern Arabian Gulf (including Kuwait), considering ichthyoplankton ecology and species composition, feeding ecology of larval fish, zooplankton abundance and species composition, including benthic copepods and pollution. Furthermore, a parallel study of adult fish in the vicinity will aid in understanding larval abundance and presence in relation to spawning time of their adult counterparts. In addition, a detailed study on the reproductive development of
various mollusks in relation to season and to compare in details with the planktonic stages is needed since their larvae constitute an important feeding component for larval fish, especially in that area where they are located.

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Author’s Contributions

M. Ali: Specialized researcher for the study area, contributed to the writing of the manuscript.

M. Al-Mutairi: Project coordinator.

M.N.V. Subrahmanyam: Specialized researcher for the study area, contributed to the writing of the manuscript.

Sasini Isath: Laboratory assistant for the physical and chemical aspects.

Mohammad A. AlAwadi: Project researcher.

P. Niranjan Kumar: GIS and topography specialist.

Khaled Al-Hebini: Field sampling specialist.

Samira A.S. Omar: Project initiator and the Director General of Kuwait Institute for Scientific Research.

Ethics

Authors declare that there is no conflict of interests.

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