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First record of *Nais elinguis* Müller, 1773 (Annelida: Oligochaeta: Naididae), Spatio-temporal patterns of its population density and biomass production along two estuaries in the South Caspian Sea, Mazandaran Province, Iran

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Abstract

The cosmopolitan oligochaete worm, *Nais elinguis,* is common to fresh and brackish water habitats. This species was found while investigating the limnology of two rivers alongside the Iranian coasts and has not been reported in the Iranian freshwater fauna and Caspian Sea before. *N. elinguis* was collected bimonthly from Cheshmehkileh and Sardabrood estuaries using a Van Veen grab (0.03 m^2) and Surber (0.1 m^2, 0.2 mm-mesh size) with three replicates from 6 stations from November 2014 through September 2015. The results of temporal distribution showed that the highest and lowest density and biomass of this species were in January (242.2±84.45 ind m^{-2} and 0.105±0.035 g m^{-2}) and in September (33.35±23.5 ind m^{-2} and 0.010±0.006 g m^{-2}) respectively which were significantly different (p<0.05). Among the sampling stations, station (S1) within the river with freshwater showed higher density and biomass (273.5±87.35 ind m^{-2} and 0.116±0.036 g m^{-2}) than riverine brackish station (S2) within the mouth (39.8±22.6 ind m^{-2} and 0.013±0.006 g m^{-2}) and marine brackish station (S3) (0±0 ind m^{-2} and 0±0 g m^{-2}) which were significantly different (p<0.05). Density and biomass of this species in Cheshmehkileh River and estuary were more than that in Sardabrood. A significant correlation (p<0.05) between the density and biomass of *N. elinguis* with environmental variables was found.

Keywords: Cheshmehkileh, Sardabrood, Estuaries, *Nais elinguis*, First record.

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Introduction

Aquatic oligochaetes are one of the main groups of macroinvertebrates and include about 1,100 species of 13 families (Martin *et al*., 2008) and have a worldwide distribution. They commonly inhabit sediments of rivers, streams, lakes, marshes, ponds, springs and ground-waters (Alves and Lucca, 2000; Wetzel *et al*., 2000; Collado and Schmelz, 2001; Montanholi-Martins and Takeda, 2001) showing that this species has been adapted to a very wide variety of habitats and environments, such as freshwater, brackish or sea water. In addition, certain Oligochaeta species are abundant in organically polluted waters because of the lack of competition and an abundant food supply coupled with a tolerance to reduced oxygen conditions (Brinkhurst and Jamieson, 1971); the reason why they are considered as an interesting bioindicator of water quality (Pinder, 1986). Most Species of naidid worms are cosmopolitan, occurring throughout the world (Wetzel *et al*., 2000) and due to their great ability to swim, they may have eyes, and are capable of exploring benthic habitat, (Erseus and Gustarsson, 2002) such as aquatic macrophytes (Alves and Gorni, 2007), mosses and liverworts; (Gorni and Alves, 2007), filamentous algae (Armendariz, 2000), sponges (Corbi *et al*., 2005), odonate larvae (Corbi *et al*., 2004) and gastropod mollusks (Gorni and Alves, 2006). Naididae is a large group of freshwater oligochaete (Milbrink, 1987; Chapman, 2001; Smutna *et al*., 2008), while 238 Naididae species are known worldwide (Martin *et al*., 2008) of which approximately 26 species belong to the genus *Nais*. Cheshmehkileh of Tonekabon and Sardabrood of Chalus Rivers are the most important, mountainous and permanent water rivers running from high elevation to the sea (southern waters of Caspian Sea). These rivers are important for the reproductive migration of two indigenous valuable fisheries species *Salmo caspius* (Caspian Salmon) and *Rutilus frisii* (Caspian Kutum) as well as for other migratory fish. For these reasons, the estuary of these rivers is regarded as a 'Protected Area' and is conserved by the Iranian Department of Environment.

Among studies on aquatic macroinvertebrates fauna in Iran, oligochaetes have been identified at the family level, and a few studies have been carried out at the species level. Besides, some work has been done regarding oligochaeta in Iran such as Stephenson (1920), Egglishaw (1980), Pourang (1996), Aliyev and Ahmadi (2010), Ahmadi *et al.* (2011, 2012), Ardalan *et al.* (2011), Basim *et al.* (2012), Jablonska and Pesic, (2014) and Nazarhaghighi *et al.* (2014). Based on these studies,, 23 species of aquatic oligochaetes have been reported from inland waters of Iran so far, 11 species from Naididae and only 2 species from the genus *Nais*: *N. Communis* (Stephenson, 1920) and *N. Pardalis* (Nazarhaghighi *et al*., 2014).

The aim of this study was to introduce *Nais elinguis* from Iran for the first time and its distribution pattern and density combined with some abiotic factors of their environments.

Materials and methods

The Cheshmehkileh is a mountainous and permanent river in the north of Iran (Mazandaran Province), with its origins in the Alamot region (north of central Alborz mountains) with a total length of approximately 80 km, average annual discharge of 55 million m^3, average slop of 6.5 % and basin area of 1200 km^2, entering the Caspian Sea in Tonekabon city. The Sardabrood is another mountainous and permanent river in the north of Iran (Mazandaran Province), originating in the Kelardasht region (north of central Alborz mountains) with a total length of approximately 67 km, average annual discharge of 100 million m^3, average slop of 6.4 % and basin area of 450 km^2, discharging into the Caspian Sea in Chalus city.

This study was carried out between November 2014 and September 2015 and random sampling was done at six stations with three replicates for each sampling along each river bimonthly (Fig. 1 and Table 1). Sampling was done using 0.03 m^2 Van Veen grab for soft sediments at the estuary area and 0.1 m^2, 0.2 mm-mesh size Surber for inner parts of the river with pebbles.

In total, 216 sediment samples (72 biotic and 144 abiotic) were collected. The samples were fixed *in situ* using a 5% formalin solution. In the laboratory, sediments were sieved through mesh sizes of 1, 0.5 and 0.25 mm and specimens were preserved in 70% ethanol and then sorted and counted under a stereomicroscope (Nikon SMZ800, Japan) and eventually the wet weight of worms was measured using a digital balance (0.0001 g, Mettler Toledo, AB204-N). For identification at species level, worm specimens were mounted on glass slides in Amman's lactophenol clearing agent (Smith, 2001) and covered by a coverslip and left for several hours to a day or two, and then for observation of setae and other details, a microscope was used (Nikon E200 & Nikon DIGTAL SIGHT DS Camera, Japan). The main identification keys used were Pinder (2010), Krieger and Stearns (2010), Smith (2001), Pinder and Brinkhurst (1994), Brinkhurst and Wetzel (1984), Brinkhurst (1986, 1971a,b).

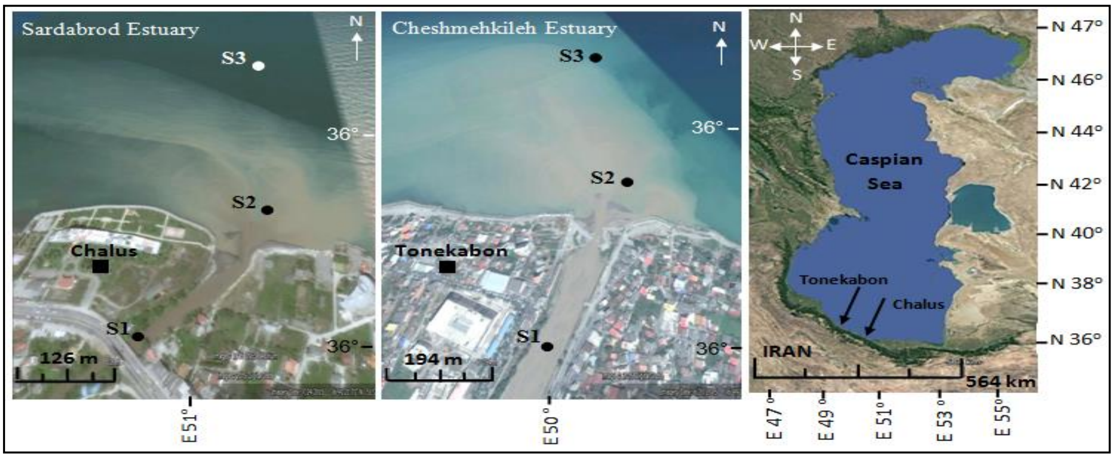

Figure 1: Sampling areas.

In this study, environmental variables such as temperature and salinity in water and TOM and grain size in sediments were measured. Alongside with sampling biota, three sediment replicates were taken at each station for grain size and TOM analysis.

Table 1: Characteristics of sampling stations.

Station	Latitude	Longitude	Sampling location	Water type	Substratum nature
S1	N 36˚ 49' 6.3"	E 50˚ 52' 52.3"	Cheshmehkileh river	Freshwater	Gravel, Sand, Silt, Clay, Vegetation
S2	N 36˚ 49' 20.0"	E 50˚ 53' 9.3"	Cheshmehkileh river mouth	Semibrackish	Gravel, Sand, Silt
S3	N 36˚ 49' 35.9"	E 50˚ 53' 24.6"	Cheshmehkileh estuary	Brackish	Sand, Silt, Clay
S1	N 36˚ 41' 11.9"	E 51˚ 23' 55.4"	Sardabrood river	Freshwater	Gravel, Sand, Silt, Clay, Vegetation
S2	N 36˚ 41' 22.2"	E 51˚ 24' 8.7"	Sardabrood river mouth	Semibrackish	Gravel, Sand, Silt
S3	N 36˚ 41' 39.9"	E 51˚ 24' 26.3"	Sardabrood estuary	Brackish	Sand, Silt, Clay

Organic matter content was measured by the weight lost during ashing; the sediment samples were oven-dried at 80 ^{0}C for 24 h, weighed to the nearest 1 mg, ashed at 550 ^{0}C for 2 h and reweighed, and the weight was expressed as the percentage of the total weight (Wildsmith *et al*., 2011).

Grain size was analyzed by dry mechanical separation through a column of standard sieves of different mesh sizes, corresponding to classes described by Wentworth (1922): gravel (>2 mm), sand (2-0.063 mm), silt (0.063-0.004 mm) and clay (0.004-0.0002 mm). Particle sizes smaller than 0.063 mm (silt and clay) were measured by hydrometery (Densimetry) method. The relative content of the different grain-size fractions was expressed as a percentage of the total sample weight.

During sampling period, water temperature and salinity were measured *in situ* by multimeter portable HACH - HQ40d model.

The statistical analysis was performed by SPSS 22. Prior to the analysis, data were tested for normality using Kolmogorov-Smirnov test. If the data were normal, three-way analysis of variance (ANOVA) test was used to determine the relationship between the environmental and biological variables. Duncan's test (p<0.05) was then used to assess the significant differences among the stations and months of sampling. Also, relationships between the density and biomass of the *N. elinguis* and environmental variables were estimated using a Pearson's correlation coefficient (p<0.05).

Results

The systematic account and description for the described species is as follows:

Kingdom: Animalia
Phylum: Annelida Linnaeus, 1758
Class: Clitellata Linnaeus, 1740
Subclass: Oligochaeta
Order: Haplotaxida Grube, 1850
Family: Naididae Ehrenberg, 1828
Subfamily: Naidinae Ehrenberg, 1828
Genus: *Nais* Müller, 1773
Species: *Nais elinguis* Müller, 1773

Prostomium is without proboscis and most specimens have no eyes. Worms are olive gray or dark yellow with brown spots (Fig. 2A). Worms body were 1.4 to 2.9 mm in length, 0.1 to 0.3 mm in diameter and the number of segments was 12–28. Dorsal chaetae beginning in segment VI (Fig. 2B), hairs 1–3 per dorsal bundle (most worms have 2-3 hairs per bundle), 140–290 µm long, needles 1–3 per dorsal bundle (most worms have 2-3 needles per bundle) (Fig. 2E, F). The needles with two long parallel teeth, distal (upper) tooth slightly longer than proximal (lower) tooth, 35-70 µm long, with a distal nodulus (Fig. 2G). Ventral chaetae 2-5 per bundle, bifid crotchets, 40–105 µm long, with a median or slightly distal nodulus, those of II-V 4–5 per bundle and slightly longer, straighter and thinner than the rest, with upper tooth 1.5–2 longer and thinner than lower (Fig. 2D), Posterior ventral chaetae 2-3 per bundle (Fig. 2C). No sexual individuals were detected during the study period.

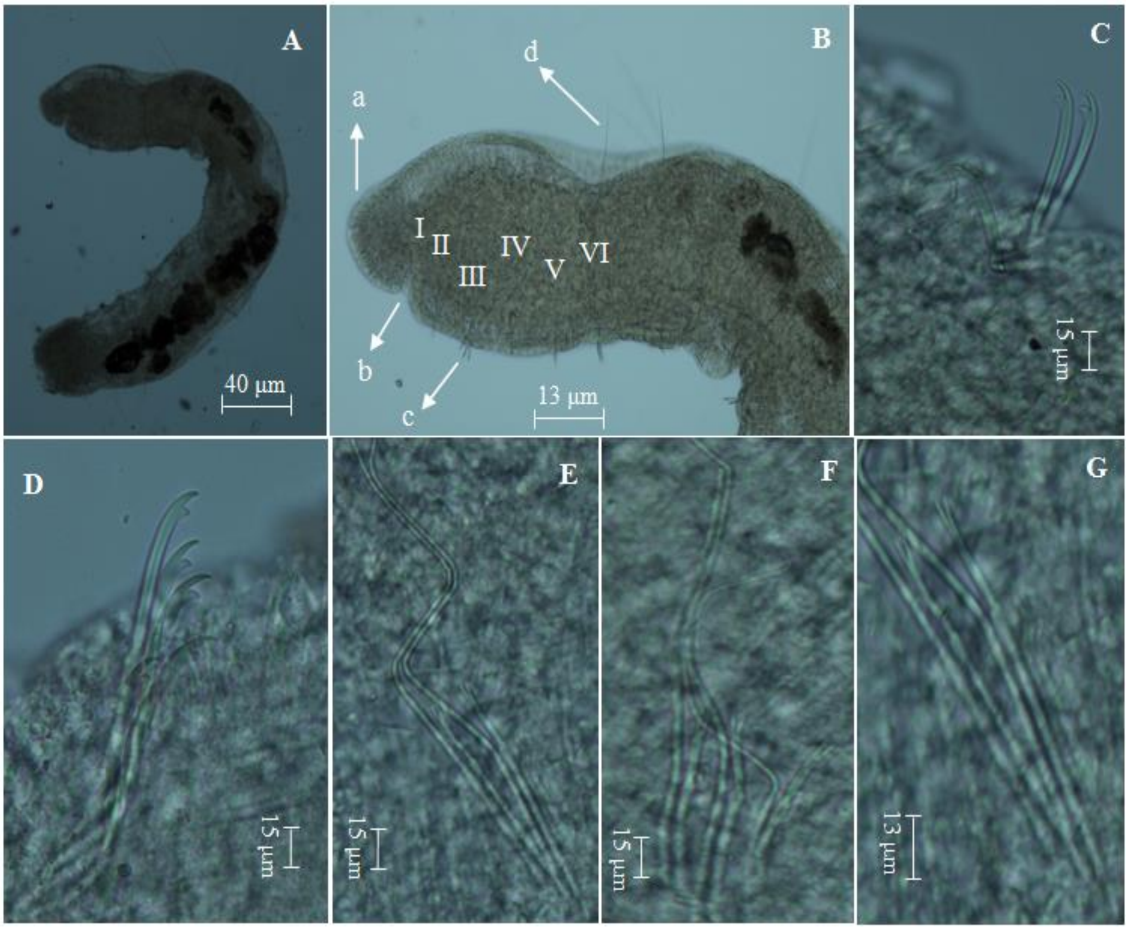

Figure 2: ***Nais elinguis*. A, General body form; B, Anterior end of the body (a: Prostomium; b: Mouth; c: Ventral bundle; d: Dorsal hair chaetae beginning in segment VI); C, Ventral chaeta bundle in posterior part of body; D, Ventral chaeta bundle in anterior part of body; E, Dorsal chaeta bundle (2 hairs and 2 needles); F, Dorsal chaeta bundle (3 hairs and 3 needles); G, 2 dorsal needles chaeta.**

In total, 526 individuals of *N. elinguis* were examined. During the present study, this species occurs in stations 1 (river) and 2 (mouth) but absent in stations 3 (estuary) in both sampling areas. Density and biomass of this species between sampling months and stations were significantly different (p<0.05), as the highest average density and biomass were observed in Cheshmehkileh (station 1) in January (613.3±165 ind m^{-2} and 0.287±0.08 g m^{-2}) respectively, while the lowest of those were in all sampling months at stations 3 of both sampling areas (0±0 ind m^{-2} and 0±0 g m^{-2}) (Table 2). Spatial distribution of *N. elinguis* between sampling stations showed significant differences (p<0.05), in both sampling areas, freshwater stations (S1) within the river (273.5±87.35 ind m^{-2} and 0.116±0.036 g m^{-2}) showed higher average density and biomass than the semibrackish station (S2) within the mouth (39.8±22.6 ind m^{-2} and 0.013±0.006 g m^{-2}) and brackish stations (S3) within the sea (0±0 ind m^{-2} and 0±0 g m^{-2}) (Fig. 4). In all sampling months station S1 had higher density and biomass than stations S2 and S3.

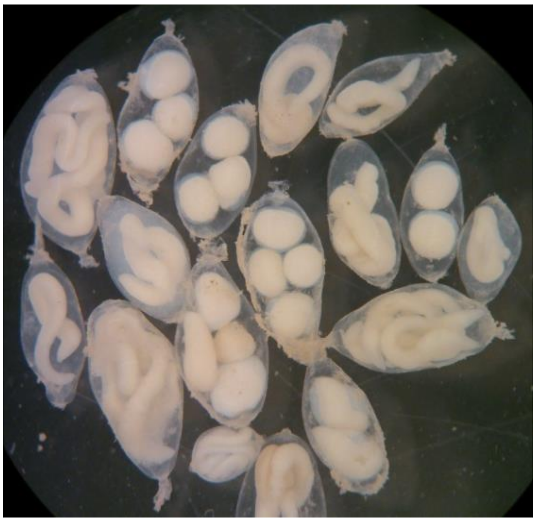

Figure 3: Oligochaeta cocoons with embryos.

No significant difference (p>0.05) was observed between the annual average density and biomass of this species for the two study areas, density and biomass of this species in Cheshmehkileh area (119.36±37.2 ind m^{-2} and 0.049±0.015 g m^{-2}) was higher than that in Sardabrood (89.5±36.07 ind m^{-2} and 0.037±0.012 g m^{-2}), respectively (Table 2).

Table 2: Density (ind m^{-2}) and biomass (gr m^{-2}) of *Nais elinguis* (average±SD)

		Cheshmehkileh			Sardabrod		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**
November	Density	cd 466.7 ± 152.7 B	bc 66.7 ± 33.33 A	-	bc 300 ± 88.2 B	a 33.33 ± 24.33 A	-
	Biomass	c 0.183 ± 0.061 C	b 0.030 ± 0.016 B	-	bc 0.124 ± 0.033 C	a 0.007 ± 0.005 A	-
January	Density	d 613.3 ± 165 C	ab 44.44 ± 30 A	-	bc 255.6 ± 101.8 B	ab 55.6 ± 41 A	-
	Biomass	d 0.287 ± 0.08 C	ab 0.014 ± 0.01 A	-	bc 0.108 ± 0.045 B	a 0.011 ± 0.007 A	-
March	Density	bc 280 ± 95.4 B	ab 11.21 ± 8.31 A	-	c 400 ± 152.8 B	-	-
	Biomass	abc 0.11 ± 0.04 B	a 0.003 ± 0.002 A	-	c 0.174 ± 0.063 B	-	-
May	Density	c 344.4 ± 51 B	c 122.2 ± 38.5 A	-	bc 233.3 ± 66.6 AB	b 100 ± 33.3 A	-
	Biomass	bc 0.137 ± 0.02 B	c 0.051 ± 0.01 AB	-	bc 0.106 ± 0.03 AB	b 0.04 ± 0.012 A	-
July	Density	ab 122.2 ± 69.4 B	-	-	ab 144.4 ± 51 B	a 33.33 ± 26.4 A	-
	Biomass	ab 0.053 ± 0.03 B	-	-	ab 0.061 ± 0.02 B	a 0.01 ± 0.005 A	-
September	Density	a 66.7 ± 46.6 A	ab 11.11 ± 9.24 A	-	a 55.6 ± 38 A	-	-
	Biomass	a 0.027 ± 0.012 CB	a 0.002 ± 0.001 A	-	a 0.013 ± 0.010 AB	-	-
Annual average	Density	315.5 ± 91.7 A	42.6 ± 19.9 B	-	231.5 ± 83 A	37 ± 25.23 B	-
	Biomass	0.132 ± 0.04 A	0.016 ± 0.007 B	-	0.1 ± 0.033 A	0.011 ± 0.005 B	-

Different letters indicate significant differences among averages (p<0.05). Capital letters indicate variation among stations (horizontal), small letters indicate variation among months (vertical).

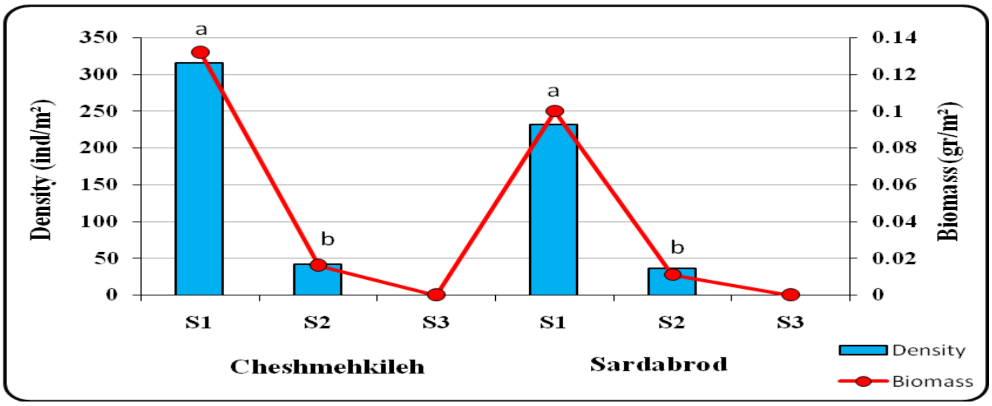

Figure 4: Annual average density and biomass of *Nais elinguis* in sampling stations Different letters indicate significant differences among averages (*p*<0.05).

Temporal distribution of this worm among sampling months was significantly different ($p<0.05$), as the highest average of density and biomass of this worm were observed in January (242.2±84.45 ind m^{-2} and 0.105 0.035 g m^{-2}) respectively, while the lowest of those were in September (33.35±23.5 ind m^{-2} and 0.010±0.006 g m^{-2}) (Fig. 5). The highest average density and biomass of this species in Cheshmehkileh area was in January and in May in the Sardabrood area, and the lowest of it in both areas was in September (Table 2).

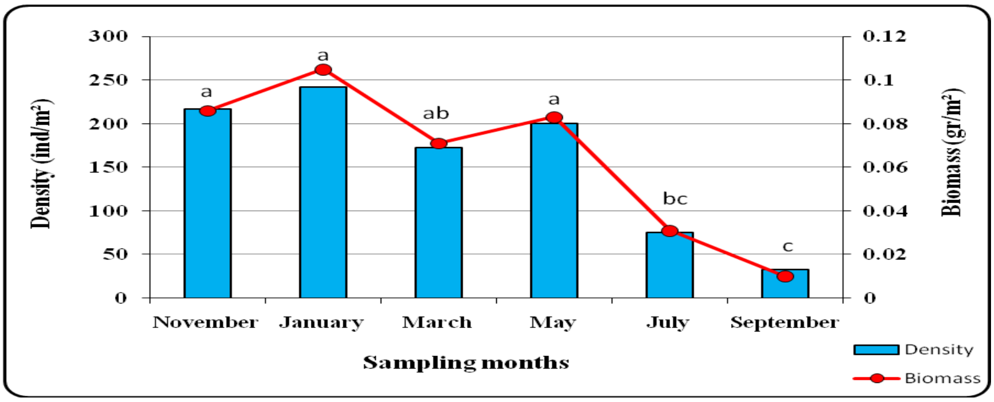

Figure 5: Density and biomass average of *Nais elinguis* among sampling months Different letters indicate significant differences among averages (*p*<0.05).

A significant correlation ($p<0.05$) between the density and biomass of *N. elinguis* with environmental variables was found (Table 3); positive correlation was found between this species and gravel, silt and clay, while an inverse correlation was found with temperature, salinity, and a TOM and sand had a negative correlation.

Table 3: Pearson's correlation coefficient between density and biomass of the *Nais elinguis* and environmental variables.

	Temperature	Salinity	TOM	Gravel	Sand	Silt	Clay
Density	- 0.397 *	- 0.635 *	- 0.039	0.280 *	- 0.557 *	0.220	0.276 *
Biomass	- 0.389 *	- 0.628 *	- 0.051	0.265 *	- 0.531 *	0.214	0.263 *

* Correlation is significant at the 0.05 level.

The lowest and highest average water temperatures were recorded as 10.2±0.1 ºC and 30±0.1 °C in Cheshmehkileh (station 1) in March and Sardabrood (staion 3) in July, respectively (Fig. 6).

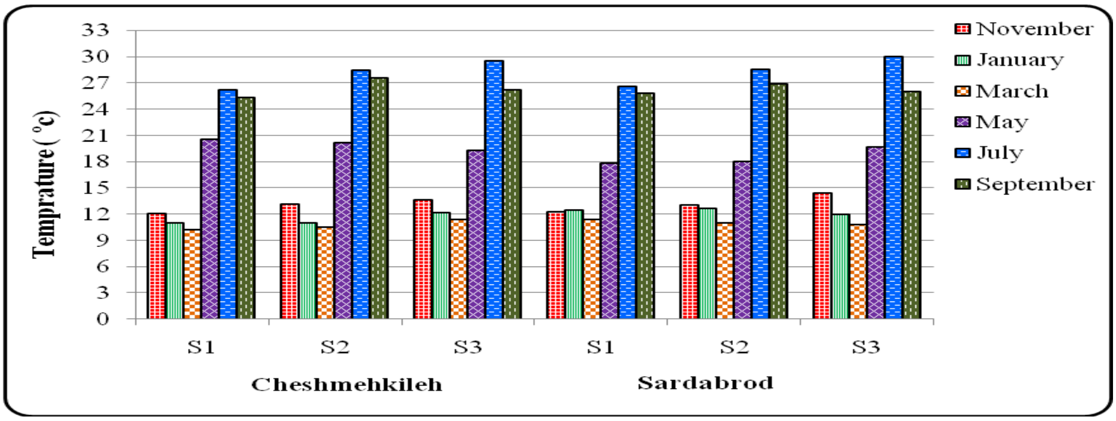

Figure 6: Annual average water temperature in sampling stations.

The lowest average salinity (0±0 ppt) was observed in all sampling months in stations 1 (fresh water) in both rivers and the highest average of it (11.61±0.01 ppt) was in Sardabrood in station 3 (brackish) in July (Fig. 7). According to the annual average, salinity level of Sardabrood area (5.77±4.71 %) was slightly higher than that in the Cheshmehkileh area (5.64±4.39 %).

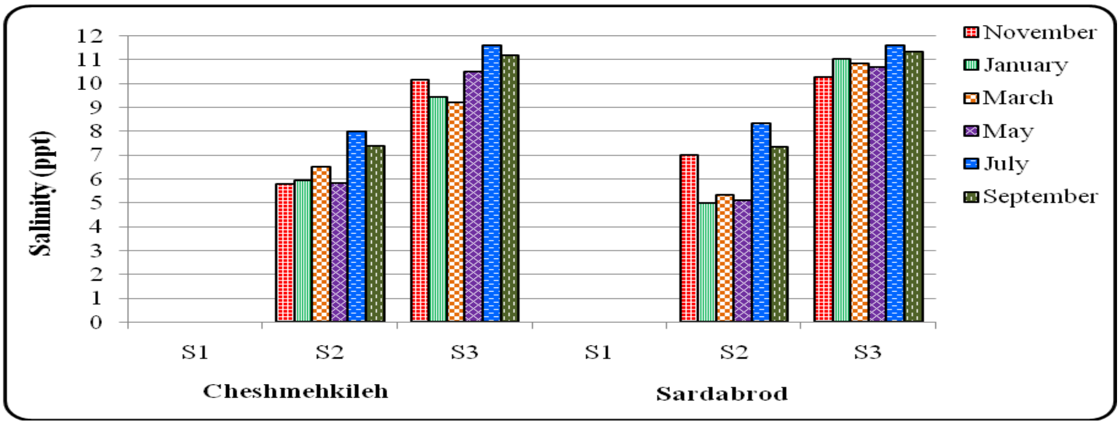

Figure 7: Annual average of water salinity in sampling stations.

Also the highest average percentage of total organic matter (TOM) (3.97±0.005 %) was measured in May in Sardabrood (station 3) and the lowest average of it (1±0.008 %) was in January in Cheshmehkileh (station 2) (Fig. 8). According to the annual average, percentage of TOM in the Sardabrood area (2.20±0.87 %) was higher than that in the Cheshmehkileh area (1.94±0.89 %).

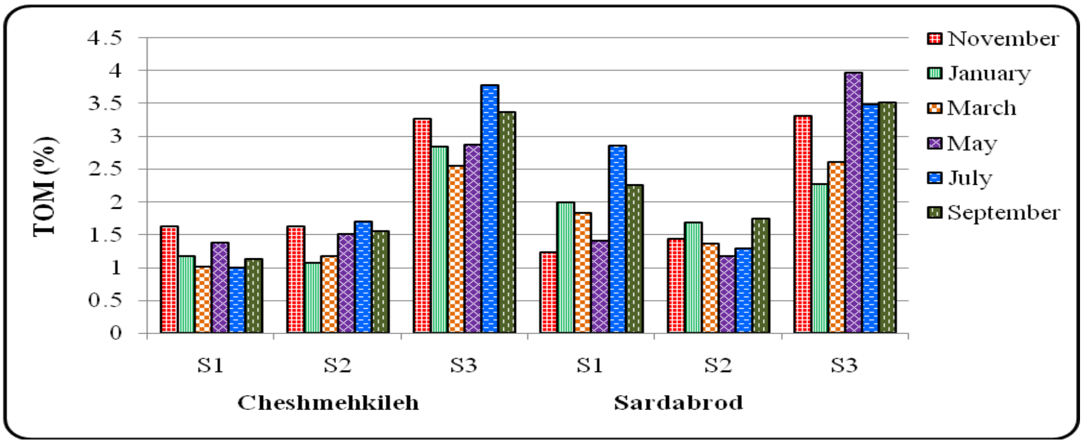

Figure 8: Annual average of TOM percentage in sampling stations.

The lowest average of gravel percentage (0±0 %) were observed in all sampling months in stations 3 in both estuaries and the highest average of it (36.55±0.05 %) was in Cheshmehkileh (staion 1) in November (Fig. 9). According to the annual average, gravel percentage in the Cheshmehkileh area (14.73±15.01 %) was higher than that in the Sardabrood area (11.51±11.08 %).

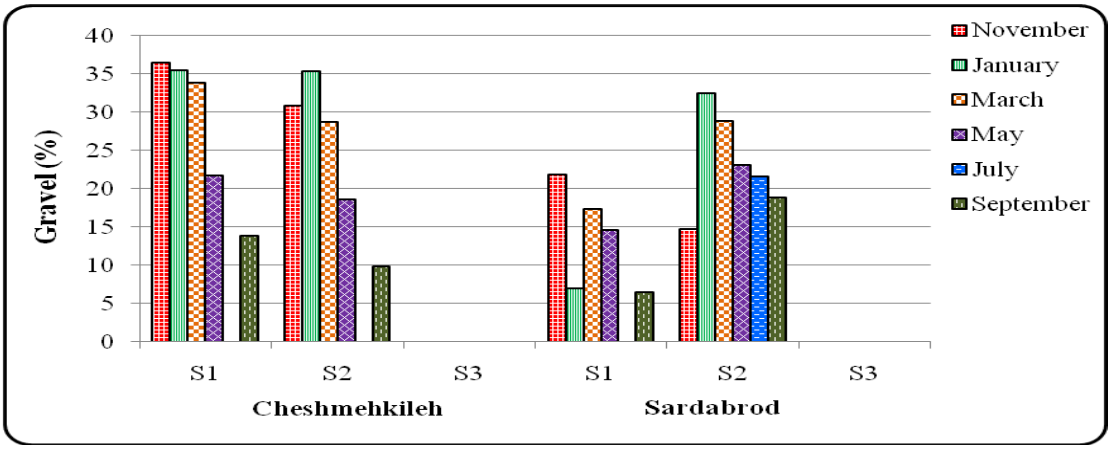

Figure 9: Annual average of gravel percentage in sampling stations.

The lowest average of sand percentage (42.81±1 %) was observed in Cheshmehkileh (station 3) in July and the highest average of it (86.46±1 %) was in Sardabrood (staion 3) in July (Fig. 10). According to the annual average, sand percentage in the Sardabrood area (70.28±9.55 %) was higher than in the Cheshmehkileh (66.82±13.72 %) area.

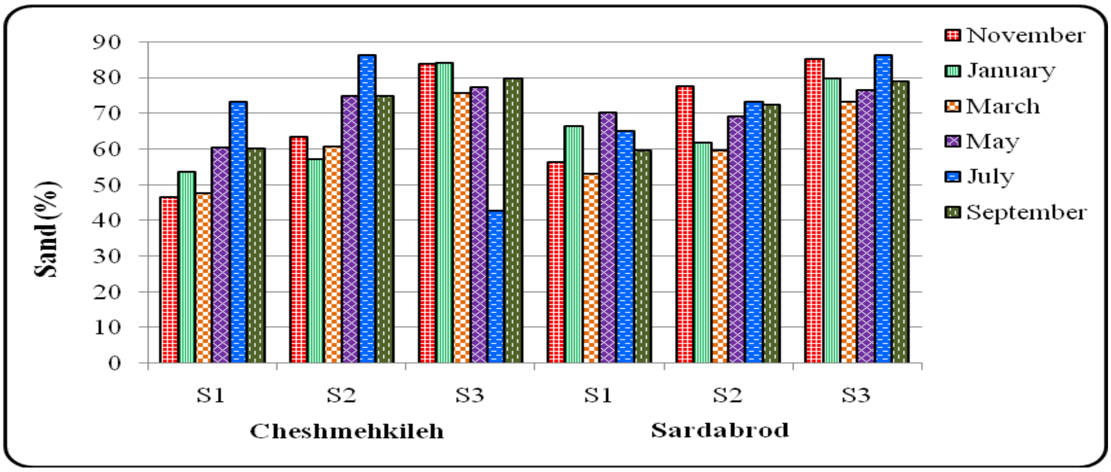

Figure 10: Annual average of sand percentage in sampling stations.

The highest average of silt percentage (38.44±1 %) was measured in Cheshmehkileh (station 3) in July and the lowest average of it (3.46±1 %) was in Sardabrood (station 2) in January (Fig. 11). According to the annual average, silt percentage in the Cheshmehkileh area (12.19±7.06 %) was slightly higher than in the Sardabrood (12.07±6.36 %) area.

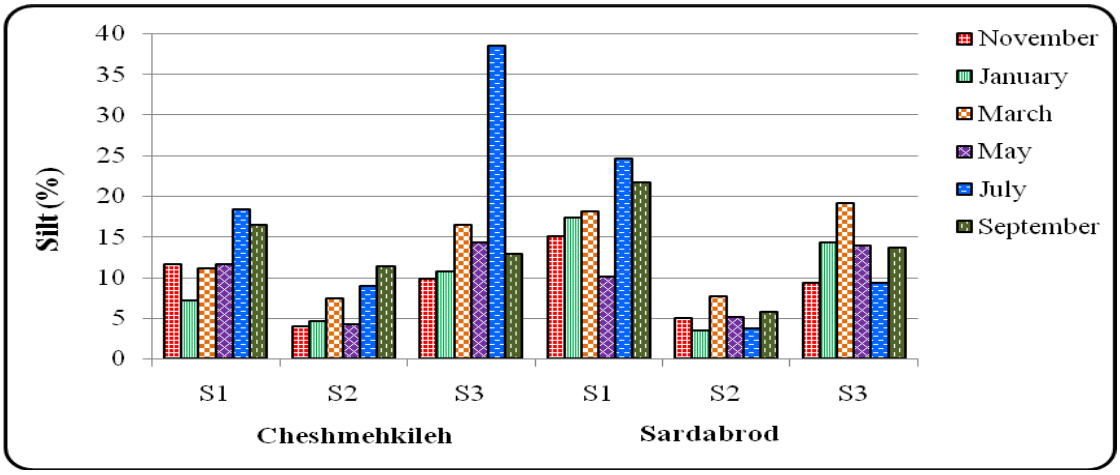

Figure 11: Annual average of silt percentage in sampling stations.

The highest average of clay percentage (18.7±1 %) was observed in Cheshmehkileh (station 3) in July and the lowest average of it (1.28±1 %) was in Sardabrood (station 2) in July (Fig. 12). According to the annual average, clay percentage of the Cheshmehkileh area (6.25±3.86 %) was slightly higher than the Sardabrood area (6.10±3.42 %).

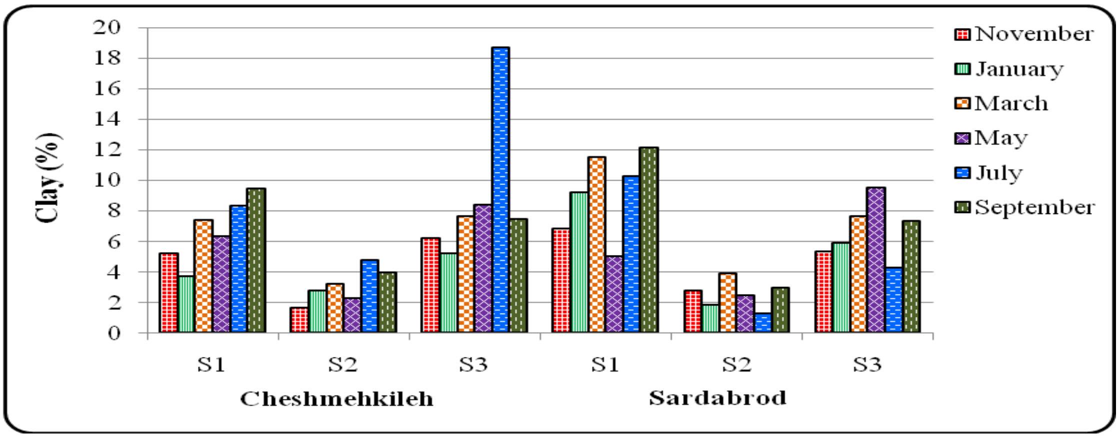

Figure 12: Annual average of clay percentage in sampling stations.

According to the grain size, stations 3 in both estuaries had finest sediments while stations 1 and 2 showed the coarsest sediments.

Discussion

During one year sampling in Cheshmehkileh and Sardabrood estuaries, 526 individuals of *Nais elinguis* were obtained. This species was recorded for the first time from Iran. This paper updated a short checklist of Iranian aquatic oligochaetes to 24 species (for Naididae to 12 species and for *Nais* genus to 3 species). In Table 4, the identified species of Naididae from Iran until now are listed and *N. elinguis* was not on the list. In this study, *N. elinguis* was a permanent inhabitant during all periods of sampling.

Table 4: List of identified Naididae species from Iran

	Species	Reference
1	*Aulophorus furcatus* Oken, 1815	Ahmadi *et al.*, 2012
2	*Chaetogaster diastrophus* Gruithuisen,1828	Stephenson, 1920
3	*Chaetogaster limnaei* Baer,1827	Stephenson, 1920; Jablonska and Pesic, 2014
4	*Dero dorsalis* Ferroniere, 1899	Jablonska and Pesic, 2014
5	*Dero digitata* Müller, 1773	Nazarhaghighi *et al.*, 2014
6	*Nais communis* Piguet, 1906	Stephenson, 1920
7	*Nais pardalis* Piguet, 1906	Nazarhaghighi *et al.*, 2014
8	*Ophidonais serpentina* Müller, 1774	Ardalan *et al.*, 2011; Nazarhaghighi *et al.*, 2014
9	*Pristina breviseta* Bourne, 1891	Jablonska and Pesic, 2014
10	*Stylaria lacustris* Linnaeus, 1767	Stephenson, 1920; Aliyev and Ahmadi, 2010; Ahmadi *et al.*, 2012; Nazarhaghighi *et al.*, 2014
11	*Slavina appendiculata* dUdekem, 1855	Nazarhaghighi *et al.*, 2014

According to the results of this study, *N. elinguis* strongly peaked at the initial cold period (from November to May) and fell at the time of summer solstice, whereas density and biomass of this species show a significant negative correlation (p<0.05) with water temperature (Table 3), also, the peak (higher mean numbers) appeared to link to the rather instable river flow regime (the first spate sampled) and the time of strong discharge fluctuations. In fact *N. elinguis* is a swimming naidid, occurring in superficial stony-sandy sediments of Cheshmehkileh and Sardabrood estuaries.

However higher densities of *N. elinguis* from November to March were initially sustained by asexual reproduction (accelerated paratomy) (Loden, 1981). The shift of asexual to sexual reproduction entails cocoon production that allows survival during environmental stress or adverse conditions (Learner *et al*., 1978; Loden, 1981) remaining during the rest of the year until December. Coincidence of active mature individuals of *N. elinguis* in the winter and spring population explosion might be also explained as a dispersal strategy. *N. elinguis* might therefore be classified as species intolerant of "late spring-summer-early autumn conditions in Cheshmehkileh and Sardabrood estuaries. According to Learner *et al*. (1978), Paoletti and Sambugar (1984), Martinez-Ansemil and Collado (1996) and Juget and Lafont (1994), spring is a key period in the life cycle of *N. elinguis* in running waters. This temporal pattern was not similar to those observed on the Danube River in May (Martinovic-Vitanovic *et al*., 2007), or by Tas *et al.* (2011) (the highest density of *N. elinguis* in June).

The naid oligochaete *N. elinguis* was only observed in station 1 and 2, higher in fresh water than brackish ecosystems ($p<0.05$), in both Cheshmehkileh and Sardabrood estuaries, while commonly found around the world in freshwater, tidal freshwater, and sometimes in brackish environments, it is rarely observed in higher salinity like marine coastal waters (Brinkhurst, 1982; Verdonschot, 1999). However, *N.elinguis* has been found in salinities up to 18 ppt (Bagge, 1969), brackish water habitats (Davis, 1982; Pinder and Brinkhurst, 1994; Yildiz *et al*., 2007), saline habitats (Pinder and Brinkhurst, 1994) and estuaries (Timm and Martin, 2015).

Substrates in both rivers in the Freshwater sampling stations (S1) were covered with vegetation in the littoral and some central zone, but in the brackish stations substrate (S2 and S3), vegetation covers were absent. *N. elinguis* is a tolerant and ubiquitous species, mostly abundant in flowing streams with abundant algal crops resulting from high nutrient input (Nijboer *et al*., 2004; Martinovic-Vitanovic *et al*., 2007) concluding that the main factors determining Naididae distribution and abundance are the nature of substrate and the presence and type of vegetation, as periphyton (vegetation habitat) offering shelter for their populations. Jablonska (2014) reported that *N. elinguis* was the dominating species in periphyton on concrete blocks. Arslan *et al*. (2014) also reported that most of the naidine taxa were found in Çatören Dam Lake stations, which contain a high density of aquatic vegetation.

Most naidid species are grazers, positively correlated with periphyton (Schenkova and Helesic, 2006); thus the abundance of the Naididae depends on the amount of periphyton (Baturina *et al*., 2014). Verdonschot (2001) found that *N. elinguis* had no preference to any substrate type. Szczesny (1974) concluded that *N. elinguis* was a dominant species in the polluted section of a mountain river on natural, stony bottoms. Likewise, other studies found that this species occurred in fast flowing, polluted streams with stony bottoms (Learner *et al*., 1978). Most species of *Nais* genus live in areas where the bottom is covered by sand, gravel or organic matter covered by aquatic plants (Lee and Jung, 2015).

Needle Naidid is a eurytolerant oligochaete and is the numerically dominant oligochaete in small estuaries. Beyond being the most abundant in Cheshmehkileh and Sardabrood estuaries, *N. elinguis* was also the most responsive organism to the environmental differences between the two estuaries and throughout the year. The lower abundance of *N. elinguis* in the lower reaches of both estuaries is indicative of reduced environmental stressors in the lower reaches. As was expected, the silt-clay composition of the sediments of Cheshmehkileh and Sardabrood estuaries did appreciably change during the study period especially in July and May and silt-clay percentages of Cheshmehkileh were approximately twice the amount found in Sardabrood. The trend of more silts and clays in the lower reaches was also noted by Lerberg *et al.* (2000); Mehdipour *et al.* (2018) and is thought to be function of particle settling rate. The absence of detectable patterns in the temporal and spatial measures of TOM of Cheshmehkileh and Sardabrood estuaries, similar to Sanger *et al.* (1999); Ghorbanzadeh Zaferani *et al.* (2017), likely indicates a relatively variable input of refractory organic material from the uplands to the sediments of the estuaries.

While there were no strong estuary-to-estuary differences in the water composition, there were significant differences in the sediment silt-clay and TOM range, and flushing rates between Cheshmehkileh and Sardabrood estuaries. These differences were a function of land cover and orientation between the two estuaries. Compared to Sardabrood estuary, Cheshmehkileh had a greater flushing rate, greater salinity fluctuation over annual cycle, which combined to create a more stressful environment in Cheshmehkileh estuary with less particulate organic matter (Fig. 8) as a food source for the benthos. These differences are most clearly reflected in the greater abundance of *N. elinguis* in Cheshmehkileh estuary compared to the Sardabrood estuary. Differential predation pressure could also be invoked to explain the differences in the abundance of *N. elinguis* between the estuaries, but the abundance of fishes was greater in Cheshmehkileh estuary than in Sardabrood during the autumn and winter (unpublished data). The greater macrobenthic abundance in Cheshmehkileh estuary in spite of the marked difference in predator abundance, strongly suggests that food supply, periphyton, and sediment quality had a greater effect on *N. elinguis* in these estuaries than predation pressure.

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