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Directional fluctuating asymmetry in certain morphological characters as a pollution indicator: Tigris catfish (*Silurus triostegus*) collected from the Euphrates, Tigris, and Shatt al-Arab Rivers in Iraq

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Abstract. The relationship between pollution levels in river sediment and fluctuating asymmetry of resident silurid fish species, *Silurus triostegus* Heckel, was assessed. Eight bilateral body proportions were analyzed in 1,500 fish from eight river locations. Sediment pollution was measured based on the analysis of heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) and organochlorine pesticides (DDT, DDD, DDE, chlordane, dieldrin, and lindane). The mean quotient approach (mERMq) was used to characterize sediment toxicity, which ranged from low to moderate levels for heavy metals and from low to severe for organochlorides. Variation was noted in the measurements of fish body morphometrics among the sampling locations, which suggested responses to local environments. Levels of asymmetry were positively correlated with both organochlorine pesticides and heavy metals across locations. These results suggest that fish asymmetry variations could be useful for estimating stress caused by organic toxicity based on the mERMq approach.

Keywords: Siluridae, morphology, morphometric characters, Iraq, pollution, bilateral asymmetry

Introduction

Aquatic environments in the vicinities of large cities are exposed to a wide range of human activities. Organisms living in these areas are subjected to myriad changes stemming from agricultural, industrial, and engineering projects and other human activities associated with growth and industrialization (Haedrich 1983). Determining the future status of biological systems depends on knowing the importance of biological effects of pollution on organisms, populations, and communities and envisaging the reactions of these organisms to environmental changes (Lajus et al. 2015). These effects can be assessed several different ways depending on the types of biological system and parameter and on the focus of particular research (Newman 2014).

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Organisms must have developmental stability to be able to follow certain developmental pathways. When instability occurs, organisms become unable to adapt to their environments (Zakharov 1989). Fluctuating asymmetry is one of the best measures of developmental instability (divergence from perfect morphological symmetry), which is why the fluctuating asymmetry index has been an increasingly attractive substitute for fitness and a measure of the effect of various environmental pressures since the late 1980s and early 1990s (Palmer and Strobeck 1986, Zakharov 1989, Parsons 1990, Graham et al. 1993). With stress, fish lose energy, which is important for growth and reproduction, and under stress developmental instability can increase (Parsons 1990, Hoffmann and Parsons 1991, Graham et al. 2010, Lajus et al. 2014).

Different kinds of heavy metals and organochlorine pesticides can find their ways into the bodies of fish or any other aquatic organisms through the food they ingest. These compounds are found in the immediate environment they inhabit including water, sediments, or the bodies of their prey (Řxnevad et al. 1995, Allenbach et al. 1999, Polak et al. 2002, Lajus et al. 2003, Ambo-Rappe et al. 2008, El Morhit et al. 2009, Lajus et al. 2015). High asymmetry index values are mainly an indication of the effects of heavy metal and organochlorine pesticide pollutants mainly on fish stress. This has been observed in many studies on different organisms (Valentine and Soule 1973, Zakharov and Ruban 1985, Zakharov et al. 1996, Green and Lochman 2006, Allenbach 2011, Lajus et al. 2014, Esin 2015).

The silurid *Silurus triostegus* Heckel is suitable for this study because it inhabits areas with a wide pollution gradient and is locally resident and a bottom feeder, which means it has direct exposure to sediment pollution, and it also possesses a sufficient number of convenient morphological characters for accurate asymmetry analysis. *S. triostegus* is distributed throughout all the rivers, lakes, and marshes of Iraq and neighboring Iran, Turkey, and Syria (Najafpour 1997, Abdoli 2000, Esmaeili et al. 2010), and it is reasonably abundant and plays a significant role in communities (Hussain and Ali 2006).

Individuals of this species prey on local benthic fish and invertebrates and therefore, it is a convenient bioindicator species (Coad 2014).

Several studies concerning the presence of pollutants in water, sediments and organisms were performed throughout Iraq. Metals and other pollutants are among these pollutants because they bioaccumulate in tissue of local animals (Scanes and Roach 1999, Alquezar et al. 2006, Lewtas et al. 2014). In all cases, metal tissue concentrations are higher in locations with elevated sediment metals. Unfortunately, no studies on record focus on the presence of heavy metals and organochlorine pesticides in *S. triostegus*. The work of Jawad et al. (2017) is the only study that undertakes the issue of the population differences of *S. triostegus* in Iraq. Jawad et al. (2017) has suggested that heavy metals and organochlorine pesticides are among the pollutants that act on the development of these elements and lead to differentiated populations of *S. triostegus*.

The objective of this study was: (1) to use fluctuating asymmetry techniques to study the effects of sediment pollution on the silurid fish, *S. triostegus* at different locations in the Tigris, Euphrates and Shatt al-Arab Rivers in Iraq as a potential predictor of environmental pollution status in fishes; (2) to show the geographical variation in the value of asymmetry, we studied variation of mean values of the asymmetry index of the chosen characters between localities.

Materials and Methods

Study area

The Tigris is one of the two great rivers that define Mesopotamia. It is 1,850 km long and rises from the Taurus Mountains in eastern Turkey. The river flows through Turkish territory before entering Iraqi territory from the north. In central Iraq near Mysan Province, the Majar al-Kabir branch of the river splits off and feeds the central marsh area (Isaev and Mikhailova 2009). The Euphrates River is the right-bank sister river to the Tigris River. It is the

most historically significant and longest river in western Asia. It is 450 km long running from its origin in southeastern Turkey and enter Syrian territories before running the Iraqi planes (Jawad et al 2017). The Shatt al-Arab River that is formed at confluence of the Tigris and Euphrates rivers in al-Qurnah, Basrah Governorate in the south of Iraq is 200 km long.

The eight sampling sites located on the three great rivers of Mesopotamia, the Tigris, Euphrates, and Shatt al-Arab, were selected because they are heavily populated and receive huge loads of industrial, agricultural, and municipal waste waters. The geographic locations and descriptions of these rivers are presented in Table 1.

Fish collection

In 1843, Heckel was the first to describe *S. triostegus* from the Tigris River at the city of Mosul, 352 km north of the capital Baghdad (Coad 2014). This species has four mandibular barbels, but it can have six of them (Haig 1952, Al-Daham and Al-Seyab 2001) when young with one pair disappearing later in the adult stage. Usually, the head is shorter than the maxillary barbels. *S. triostegus* is characterized by a strongly serrated pectoral fin spine with serration directed toward the inner surface. The lower jaw is longer than the upper with recurved teeth in both jaws. This species differs from *Silurus glanis* L. in that it has longer, robust teeth, lower jaw teeth that are exposed when the mouth is closed, a coarsely serrated pectoral fin spine, a lighter color, and maxillary barbels that are equal to head length. In *S. glanis* they are much longer and the eye is larger. The dorsal side of the body is pale yellow speckled with brown and black, and the abdomen is white spotted with black.

Fish samples were obtained from fishers operating at the sampling sites (Fig. 1). Gillnets (200 m x 1.30 m with different mesh sizes including 25, 40, and 50 mm)

and cast nets (6 m in diameter, 20 mm mesh size) were used by the fishers to catch the fish. Fish samples were collected from the Tigris River locations in August 2007 and from the Euphrates and Shatt al-Arab river locations in July 2007 (Table 1). The depth range at all sampling sites was 0.5–2.4 m. The total number of specimens of the freshwater catfish *S. triostegus* studied was 1,500; these specimens ranged in total from 200 to 500 mm and in weight from 400 to 3,500 g.

Selection of characters

The eight metric characters chosen to assess fluctuating asymmetry (Fig. 1) have been used previously in other fish studies (Hechter et al. 2000, Lucentini et al. 2002, Jawad et al. 2010). They are as follows: (1) preorbital length (ProL) measured from the tip of the mouth to the anterior edge of the orbit; (2) postorbital length (PosL) measured from the posterior edge of eye to the edge of the operculum; (3) eye diameter
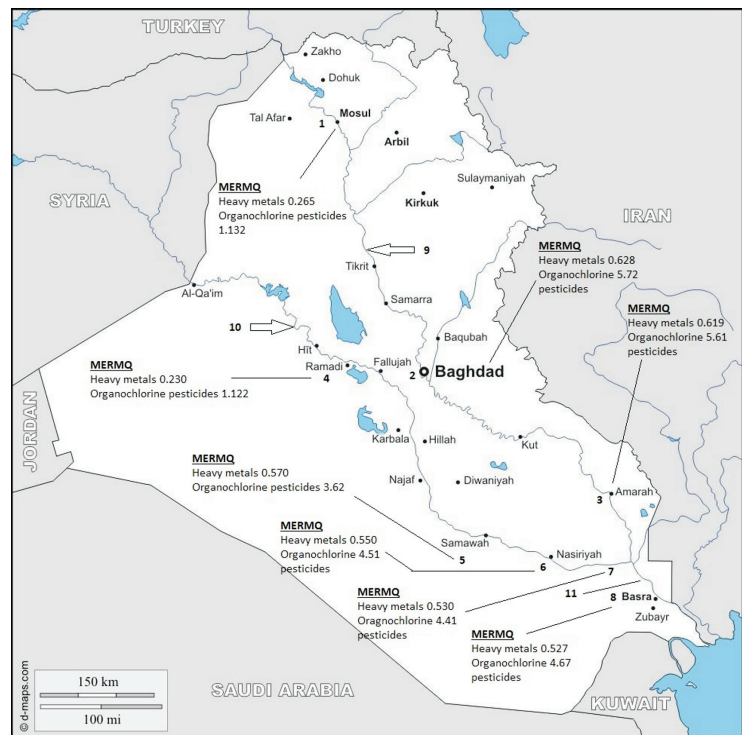

Figure 1. Map showing sampling sites of *Silurus triostegus* in the Tigris, Euphrates, and Shatt al-Arab rivers in Iraq. 1 – Mosul; 2 – Baghdad; 3 – Maisan; 4 – Ramadi; 5 – Samawa; 6 – Nasiria; 7 – Al-Hammar Marsh; 8 – Basrah; 9– Tigris River; 10 – Euphrates River; 11 – Shatt al-Arab River.

Table 1
Coordinates and bottom type of eight sampling sites localities in the Tigris and Euphrates rivers in Iraq.

| Localities	Coordinates	Description of the area
Mosul	36.34°N 43.13°E	Mainly silt and rocky bottom with plenty of aquatic plants
Baghdad	33°20'N 44°26'E	Mainly silt and pebbles with plenty of aquatic plants
Al-Amarah	1°54'N 47°2'E	Mainly silt rich with large aquatic plants
Ar Ramadi	3°25'11"N 43°18'45"E	Mainly rocky with fine silt and fewer aquatic plants
As Samawah	31°19'N 45°17'E	Mainly silt with small rocks and plenty of aquatic plants
An Nasiria	31°03'N 46°16'E	Mainly silt and plenty of aquatic plants
Al-Hammar Marsh	30°46'0"N 47°3'0"E	Mainly silty and rich with large aquatic plants and algae
Basrah	30°30'N 47°49'E	Mainly silty and rich with large aquatic plants and algae
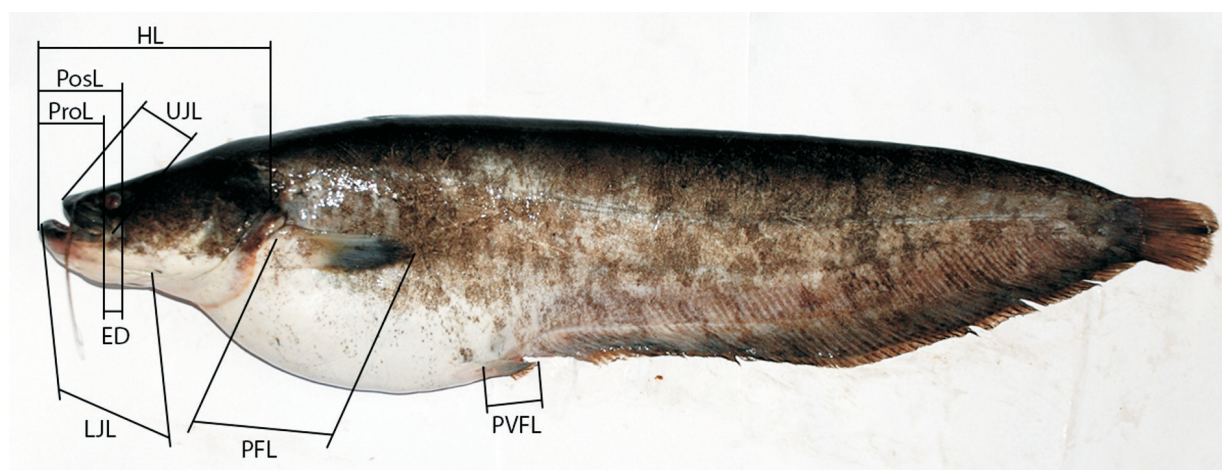
Figure 2. Bilateral body characters of *Silurus triostegus*. Preorbital length (ProL); postorbital length (PosL); eye diameter (ED); head length (HL); upper jaw length (UJL); lower jaw length (LJL); pectoral fin length (PFL); pelvic fin length (PVFL).

(ED) measured from the anterior edge of the orbit to the posterior edge of the orbit; (4) head length (HL) measured from the anterior tip of the mouth to the edge of the operculum; (5) upper jaw length (UJL) measured from the anterior symphysis of the left and right upper jaws to the posterior bony edge of the upper jaw; (6) lower jaw length (LJL) measured from the anterior symphysis of the left and right parts of the lower jaw to the posterior bony edge of the lower jaw; (7) pectoral fin length (PFL) measured from the origin of the fin to the tip of the longest ray; (8) pelvic fin length (PVFL) measured from the origin of the fin to the tip of the longest ray (Fig. 2). The characters were measured to the nearest 0.1 cm using digital calipers.

Chemical analyses of organochlorine pesticides and heavy metals in surficial sediments

Since *S. triostegus* is a bottom debris feeder, the level of concentration of organochlorine pesticides and heavy metals were assessed in the sediments rather than in the water. Concentrations of the heavy metals (cadmium, chromium, copper, lead, nickel, and zinc) in the sediments were determined with flame atomic absorption spectrometry (Perkin–Elmer 3300, USA; detection limit 0.001–0.020 mg kg^{-1}) after sample digestion. The digestion method used was that reported by the US Environmental Protection Agency with minor modifications. The samples were air-dried, disaggregated using a mortar and pestle and passed through a 2 μm mesh sieve, dried at

105°C for 24 h, and digested as follows: 15 mL concentrated ultrapure nitric acid was added to the sample (2.0 g) and heated to 160°C for 1 h; then the vessel was cooled to room temperature and 10 mL ultrapure concentrated hydrochloric acid was added and the flask was heated to 160°C for 1 h. The samples were dried and heated in a model VO914SA Lindberg Blue M Vacuum Oven (USA). The mixture was cooled, filtered (Whatman Grade No. 42, particle retention 2.5 µm), and diluted with ultrapure water to 50 ml.

Ten fish specimens from each sampling site were used to assess organochlorines and heavy metals. Samples for the analysis of organic pollutants were stored at 20°C in polypropylene containers. The methods used for the extraction, clean-up, and analysis of organic pollutants in fish were validated previously and are described briefly below (Jacobs et al. 2002).

The materials used were pesticide-grade acetone, n-hexane, acetonitrile, and diethyl ether that were obtained from commercial suppliers and used as received (Burdick & Jackson Laboratories Inc.). High-purity (> 99%) pesticide standards and related compounds were supplied by Chrompack International B. V. and used without further purification. Organochlorine pesticide residues were extracted using Florisil PR 60-100 mesh (Supelco SA) and anhydrous, granular sodium sulphate (Fisher Scientific Company) with n-hexane for a minimum of 36 h in a Soxhlet apparatus. Following cleanup extraction, they were oven dried at 130°C for 24 h (model VO914SA Lindberg Blue M Vacuum Oven, USA) prior to use. All non-volumetric glassware used was acetone-rinsed then oven-baked at 300°C for about 24 h prior to use. Volumetric glassware was sequentially rinsed with acetone and n-hexane, followed by air drying. Superficial sediments were collected from all localities studied using a clean grab sampler. Only the top layer (about 5 cm) was collected, wrapped in aluminum foil, and stored at -20°C until analysis.

The extraction procedure employed in the present study was based on the Draught Method of the Standing Committee of Analysis for determinations of organochlorine insecticides (Barceló 1993).

Twenty grams of sediment samples was placed in a pre-extracted cellulose thimble and Soxhlet extracted with n-hexane for about 24 h. At the end of this period, the extract was transferred to a storage flask and samples were further extracted with fresh solvent. The combined extracts were reduced in volume to approximately 10 ml in a rotary evaporator.

Extract cleanup prior to gas chromatographic analysis eliminates, *inter alia*, many volatile constituents in samples, which improves resolution and prolongs column life. Therefore, our extracts were cleaned by transferring them to a separating funnel and apportioning between acetonitrile/n-hexane (Mills 1961). Then, the acetonitrile was diluted with water and the residues were extracted with n-hexane that was dried over a column of sodium sulphate. However, in order to remove the unsaponified lipids, extracts were further cleaned and fractioned on active Florisil. The extract was charged to the column, and elution took place with 6% and 15% diethyl ether in n-hexane. The combined elutants were treated with activated copper to remove elemental sulphur and were evaporated to about 10 ml by in a rotary evaporator, then to exactly 1 ml with a stream of purified nitrogen.

Two model 304 Pye Unicam gas chromatographs (Canada) equipped with constant current Ni 63 electron-capture detectors and SP 4100 computing integrator (Spectra-Physics, Inc.) were used to quantify peaks and identify pesticides in chromatograms. Abate was used as the internal standard. A wall-coated open tubular (WCOT) fused silica capillary column (30 × 0.25 mm ID) with 0.22 µm film thickness coated with SE-30 (methylsilicone) (Supelco SA) was used. Operating temperatures were 220, 270, and 300°C for the column, injector, and detector, respectively. A mixture of 95 + 5% argon/methane was used as the carrier gas with a linear velocity of 75 cm s^{-1} and a split ratio of 10:1. An argon/methane mixture was also used as a make-up gas to boost the flow into the detector to 60 ml min^{-1}. A second WCOT glass capillary column, 60 m long and 0-2 mm ID with 25 µm film thickness coated with SE-54 (phenyl polymethylphenylsiloxane) was employed to determine the proportion of DDT and its

metabolites. This column was operated under temperature programmed conditions (1°C min) from 200 to 250°C with an isothermal period (10 min) at the end. Argon was the carrier gas with a linear velocity of 80 cm s^{-1} with a 95 + 5% argon/methane mixture as the make-up gas to increase the flow in the detector to 65 min^{-1}. Exactly half of the concentrated extracts were injected splitless (60s splitless period "hot needle") with the injection port at 250° C and the electron-capture detector at 300°C. Operating temperatures were 200, 220, and 300° C for the column, injector, and detector, respectively. A mixture of 90 + 10% argon/methane was used as the carrier gas at a flow rate of 30 ml min. Blanks Procedural blanks, consisting of all reagents and glassware used during the analysis, were determined periodically.

Data analysis

Probability of toxicity

The guidelines of Long et al. (1995) for probability of toxicity were followed. These consist of two parametric concentrations for each of the chemicals given in these guidelines. The lower level (effects range low, or ERL) signifies the concentration below which adverse biological effects are seldom observed and the effects range median (ERM) level signifies concentrations above which adverse biological effects are expected to occur frequently. Any value falling between the ERL and ERM parameters indicated an intermediate, often irregular, biological response. Individual chemicals were assessed for possible adverse biological effects by comparing concentrations at each site to the respective ERL and ERM values. Sediments were classified into four risk categories of low, moderate, high and severe following the scheme of Long et al. (2000).

Fluctuating asymmetry

Directional fluctuating asymmetry values and measurement errors are small and normally distributed around a mean of zero in most cases (Merilä and Bjöklund 1995). Because of the personal effect that results in errors in fluctuating asymmetry results, all measurements were executed by only one person to avoid discrepancies, and they were repeated twice. Morphological measurements can be affected by factors such as measurement conditions and technician experience (e.g., Lajus et al. 2003). Therefore, randomly selected samples were measured regardless of the order of sampling locations to avoid the effect of these factors on the results of sample comparisons.

In the statistical analysis, the square coefficients of directional asymmetry variation (CV^2_a) for meristic and morphometric characters were calculated according to Valentine et al. (1973) as:

$$CV^2_a = (S_{l-r} X\ 100/X_{l+r})$$

where S_{l-r} is the standard deviation of the signed difference, X_{l+r} is the mean of the character, which is calculated by adding the absolute scores for both sides and dividing by the sample size. To measure individual directional fluctuating asymmetry, first we standardized all absolute individual asymmetry values by sample means and variance, and then determined the average asymmetry values for all the characters for each individual.

To eliminate scaling problems associated with growth in morphometric characters (non-discrete, measurable), each measurement was divided by a conventional standardizing measurement (e.g., total length was used in the present study). Every morphometric measurement was treated in a similar manner and the squared coefficient of asymmetry was determined as before. The coefficient of directional asymmetry was calculated for both individuals within each locality and among all individuals. For each locality, the sampled individuals were divided into length classes based on total length. Specimens from the eight localities were divided into three size classes to calculate the fluctuating asymmetry value for each size class. Coefficients of asymmetry were compared among and within the eight fish populations with ANOVA tests.

Heavy metal data analysis

Statistical analyses were performed using SAS software (SAS Institute 1990). Analysis of variance (ANOVA) was performed for each of the six metals separately to test for differences among all sampling sites. Significant differences demonstrated by the ANOVA *F*-test were further tested using Tukey's honest significant difference (HSD) multiple comparison method (P < 0.05). As a measure of association between asymmetry level and the concentration of both heavy metals and pesticides, the Pearson correlation coefficient was calculated using the data from the eight sites for each of the 12 pollutants (6 heavy metals and 6 pesticides). The normality and equal variance assumptions of ANOVA were checked using residual plots. If equal variance was not achieved, the log of the data was used for the test.

Results

The results of the directional fluctuating asymmetry analysis of the eight morphological characters of *S. triostegus* are shown in Tables 2 and 3. Except for the Mosul sampling site, the asymmetry values of the eight morphological characters were higher at the Tigris River sampling sites than at those in the Euphrates River, while the values obtained at the Shatt al-Arab River sampling sites were within the ranges of the values at the Tigris and Euphrates river sites. The eight morphological characters showed higher values in fish collected from the Baghdad area and the lowest values in fish obtained from the vicinity of the city of Mosul (Tables 2 and 3).

Analysis of variance showed that the values of asymmetry for the eight morphological characters differed among the *S. triostegus* populations studied along the Tigris, Euphrates, and Shatt al-Arab rivers (P < 0.001). All eight morphometric characters exhibited the highest and lowest percentages of bilateral asymmetry at the Baghdad (90-96%) and Mosul (32-46%) sites. *S. triostegus* individuals from the eight localities were grouped into length classes (Table 3). Increasing trends in asymmetry values were noted for preorbital length, postorbital length, upper jaw length, lower jaw length, and pectoral fin length in the eight populations of *S. triostegus*.

A significant correlation between the asymmetry index and total length for the entire dataset for sample means (r = 0.658) was observed. On the other hand, multiple regression analysis that included the asymmetry index as a dependent variable and TL and Sex, which included males, females, juveniles, and fish with indefinite sex, as independent variables indicated that the asymmetry index was related to fish TL but not with sex. To check whether fish of the same size from Baghdad and both Mosul and Ramadi Cities differ in the fluctuating asymmetry index, samples from both places with the same size were prepared (60 of the largest fish from waters in the vicinity of Baghdad and all 85 fish from waters in the vicinities of Mosul and Ramadi) and the fish from Baghdad had higher fluctuating asymmetry index (P < 0.001).

Sediment pollution in the Baghdad area was notably higher than that in the other locations studied (Table 4). The mERMq values for heavy metals in the waters in the vicinity of Baghdad exceeded those in the vicinity of Mosul by nearly 2.5 fold and those in the vicinity of Ramadi by 2.7 fold. For organic pesticides the mERMq values in the waters in the vicinity of Baghdad were 5.1 times higher than those for Mosul and Ramadi. Except for the fish from the vicinity of Mosul, the heavy metal and organic pesticide mERMq values were higher in Baghdad and Mysan located on the Tigris River than those of fish from cities located on the Euphrates and Shatt al-Arab rivers (Table 4). The use of mERMq techniques indicated that the risk was low of heavy metal toxicity at the sites located near Mosul and Ramadi, while that at the other sites was moderate (Table 4). The risk of toxicity associated with organic pollution was considerably higher and was close to severe at the Baghdad and Mysan sites, while at the other sites it was low.

Overall, ANOVA indicated there were significant differences in sediment concentrations among the sites for all 12 pollutants. Sites on the Tigris River were significantly different from those on Euphrates

Table 2
Squared coefficient asymmetry (CV^2a) values and character means (X_{r+1}) of *Silurus triostegus* collected from different sampling sites on the Tigris and Euphrates rivers in Iraq

		Characters							
	Locality	Preorbital length	Postorbital length	Eye diameter	Head length	Upper jaw length	Lower jaw length	Pectoral fin length	Pelvic fin length
CV^2a	Mosul	24.56	34.7	45.3	48.3	38.3	27.9	53.8	42.5
	Baghdad	119.89	98.9	123.4	112.2	120.3	96.4	114.5	120.3
	Al-Amarah	87.98	88.5	98.4	96.4	98.3	90.4	98.5	97.7
	Ar Ramadi	55.54	65.9	77.4	79.5	87.4	77.5	87.5	89.7
	As Samawah	76.34	87.6	98.3	96.5	93.2	89.1	90.8	86.2
	An Nasiria	67.98	75.3	88.5	86.4	98.3	86.6	89.0	90.8
	Al-Hammar Marsh	77.27	87.3	96.4	94.3	92.4	74.8	84.3	89.6
	Basrah	97.90	101.2	99.4	100.2	99.4	95.4	99.7	100.3
N	Mosul	180	180	180	180	180	180	180	180
	Baghdad	190	190	190	190	190	190	190	190
	Al-Amarah	200	200	200	200	200	200	200	200
	Ar Ramadi	195	195	195	195	195	195	195	195
	As Samawah	140	140	140	140	140	140	140	140
	An Nasiria	187	187	187	187	187	187	187	187
	Al-Hammar Marsh	210	210	210	210	210	210	210	210
	Basrah	198	198	198	198	198	198	198	198
Character mean (X_{r+1})	Mosul	0.9	5.4	1.7	9.6	2.6	3.4	5.0	3.0
	Baghdad	1.0	5.5	0.4	9.8	2.7	3.0	5.2	3.2
	Al-Amarah	1.3	5.7	0.6	10.0	2.6	3.5	5.2	3.5
	Ar Ramadi	1.0	5.6	0.5	9.8	2.6	3.4	5.3	3.5
	As Samawah	1.6	4.3	0.3	7.6	1.5	2.1	4.6	2.7
	An Nasiria	1.0	5.7	0.5	10.4	2.6	3.5	5.2	3.2
	Al-Hammar Marsh	0.9	5.5	0.4	9.3	2.6	3.5	5.0	3.0
	Basrah	1.0	5.3	0.4	9.3	2.6	2.6	5.0	3.0
Individuals with asymmetry	Mosul	35%	32%	38%	40%	42%	44%	46%	43%
	Baghdad	90%	95%	94%	96%	94%	96%	94%	90%
	Al-Amarah	85%	85%	82%	85%	82%	85%	84%	80%
	Ar Ramadi	76%	78%	75%	78%	73%	74%	72%	67%
	As Samawah	76%	74%	72%	75%	71%	72%	70%	69%
	An Nasiria	77%	72%	71%	73%	73%	75%	72%	70%
	Al-Hammar Marsh	80%	76%	78%	80%	83%	87%	85%	80%
	Basrah	87%	83%	84%	83%	85%	86%	81%	79%

Table 3

Squared coefficient asymmetry (CV²a) values and character means (Xr+1) of *Silurus triostegus* collected from different sampling sites on the Tigris and Euphrates rivers in Iraq. Length group 1 = 200–300mm; length group 2 = 301–400 mm; length group no.3= 401–500 mm

	Locality	Preorbital length			Postorbital length			Eye diameter			Head length			Upper jaw length			Lower jaw length			Pectoral fin length			Pelvic fin length		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CV²a	Mosul	20.1	25.3	28.4	29.5	30.5	34.5	43.2	45.2	42.9	45.4	42.3	48.6	32.3	34.5	38.4	23.1	25.4	27.8	50.9	52.3	53.6	43.2	42.3	43.2
	Baghdad	74.2	88.4	95.3	91.5	98.4	96.4	99.8	89.9	99.9	110.2	99.9	98.3	90.4	96.3	120.0	91.8	92.7	96.2	98.5	99.8	113.2	120.0	99.4	98.5
	Al-Amarah	73.2	75.8	78.4	88.1	83.6	87.6	95.4	93.8	98.1	95.4	96.6	96.6	92.1	94.3	98.4	73.1	74.5	76.4	92.4	95.4	98.2	95.4	97.5	93.6
	Ar Ramadi	45.7	47.8	55.1	64.2	65.4	61.3	73.2	72.4	77.8	75.4	78.9	79.6	82.1	84.3	87.3	72.3	74.3	77.1	81.3	84.8	87.3	89.7	89.9	84.3
	As Samawah	69.5	70.4	76.5	85.8	82.5	87.2	93.4	92.4	98.4	95.1	96.4	95.4	89.8	91.3	93.8	82.1	85.3	89.0	85.5	88.4	90.4	86.0	86.4	85.4
	An Nasiria	60.9	63.5	67.5	73.2	70.1	75.4	86.3	82.7	88.0	86.4	84.3	86.7	92.1	95.3	98.3	80.1	83.4	86.2	83.4	85.4	89.0	89.3	90.8	90.4
	Al-Hammar Marsh	61.4	63.5	69.6	86.3	83.2	87.8	97.3	94.2	98.7	94.3	92.6	94.7	89.6	90.4	92.3	71.4	73.9	78.1	73.4	75.3	78.4	89.4	87.6	89.3
	Basrah	93.5	96.4	97.6	99.3	95.3	99.7	96.6	93.8	99.4	99.9	98.3	99.0	95.2	97.4	99.5	91.4	93.2	95.2	93.4	97.4	99.4	100	99.3	99.5
N	Mosul	60	80	40	60	80	40	60	80	40	60	80	40	60	80	40	60	80	40	60	80	40	60	80	40
	Baghdad	50	90	50	50	90	50	50	90	50	50	90	50	50	90	50	50	90	50	50	90	50	50	90	50
	Al-Amarah	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
	Ar Ramadi	70	80	45	70	80	45	70	80	45	70	80	45	70	80	45	70	80	45	70	80	45	70	80	45
	As Samawah	65	60	15	65	60	15	65	60	15	65	60	15	65	60	15	65	60	15	65	60	15	65	60	15
	An Nasiria	70	80	37	70	80	37	70	80	37	70	80	37	70	80	37	70	80	37	70	80	37	70	80	37
	Al-Hammar Marsh	90	80	40	90	80	40	90	80	40	90	80	40	90	80	40	90	80	40	90	80	40	90	80	40
	Basrah	80	75	43	80	75	43	80	75	43	80	75	43	80	75	43	80	75	43	80	75	43	80	75	43
Character mean (X_{+1})	Mosul	0.8	0.8	0.9	5.2	5.3	5.4	1.5	1.6	1.7	9.4	9.5	9.6	2.4	2.5	2.6	3.1	3.2	3.4	4.8	4.9	5.0	2.8	2.9	3.9
	Baghdad	0.9	1.0	1.0	5.0	5.1	5.1	0.3	0.4	0.4	9.6	9.7	9.8	2.4	2.5	2.6	2.8	2.9	3.0	5.0	5.1	5.2	0.2	0.3	0.4
	Al-Amarah	1.1	1.2	1.3	5.5	5.6	5.7	0.5	0.5	0.6	9.0	9.5	10	2.4	2.5	2.6	3.2	3.4	3.5	5.0	5.1	5.2	0.2	0.3	0.4
	Ar Ramadi	0.9	1.0	1.0	5.4	5.5	5.6	0.4	0.5	0.5	9.6	9.7	9.8	2.4	2.5	2.6	3.2	3.3	3.4	3.3	3.4	3.5	0.3	0.4	0.5
	As Samawah	1.3	1.4	1.6	5.5	5.5	5.6	0.2	0.3	0.3	7.4	7.5	7.6	1.3	1.4	1.5	2.0	2.1	2.1	4.4	4.5	4.6	0.3	0.4	0.5
	An Nasiria	0.9	1.0	1.0	5.6	5.7	5.7	0.4	0.5	0.5	10.3	9.9	10.4	2.4	2.5	2.6	3.3	3.4	3.5	3.0	3.1	3.2	0.3	0.4	0.5
	Al-Hammar Marsh	0.9	0.9	0.9	5.3	5.4	5.5	0.3	0.4	0.4	9.1	9.2	9.3	2.4	2.5	2.6	3.3	3.4	3.5	4.8	4.9	5.0	0.2	0.3	0.4
	Basrah	0.9	1.0	1.0	5.2	5.3	5.3	0.5	0.6	0.6	9.1	9.2	9.3	2.4	2.5	2.6	3.3	3.4	3.5	4.8	4.9	5.0	0.2	0.3	0.4
% of individuals with asymmetry	Mosul	30	35	50	35	25	45	34	43	52	34	45	48	23	35	36	10	16	19	30	34	40	32	33	32
	Baghdad	53	67	89	76	85	86	87	90	92	89	90	94	87	88	86	80	83	87	87	86	80	98	99	97
	Al-Amarah	52	63	89	78	86	90	86	88	90	86	78	77	79	85	89	54	53	60	87	78	79	85	83	87
	Ar Ramadi	23	35	78	53	65	67	67	75	74	64	63	54	65	67	78	65	67	69	32	37	40	76	74	79
	As Samawah	45	55	86	78	80	87	64	53	78	78	80	83	67	69	79	76	77	87	78	88	89	76	78	79
	An Nasiria	43	62	77	65	63	70	75	76	63	65	67	76	87	88	78	76	77	79	78	75	79	87	86	89
	Al-Hammar Marsh	54	44	78	77	77	80	87	80	79	87	79	90	67	78	80	65	66	70	65	66	69	62	66	69
	Basrah	87	89	96	89	87	85	87	88	90	88	78	98	85	86	89	87	85	88	87	88	94	98	99	99

Table 4

Concentration (μg^{-1}ml) (upper line), quotient (lower line) and mean effects range median quotients (MERMQs) of six heavy metals and six organochlorine pesticides at sample sites in the Euphrates, Tigris, and Shatt al-Arab Rivers in Iraq

Locality		Heavy metals									Organochlorine pesticides								Overall toxicity
		Cd	Cr	Cu	Pb	Ni	Zn	mERMq	Risk of toxicity	P value	Lindane	DDE	DDD	DDT	ttChlordane	Dieldrin	mERMq	Risk of toxicity	
Mosul	Mean	10	9	16	28	5	83	0.265	Low	P<0.05	1.9	2.0	3.0	2.6	1.2	2.0	1.132	Low	0.102
	Quotient	0.03	0.03	0.08	0.14	0.02	0.20				0.07	0.07	0.15	0.07	0.210	0.28			
Baghdad	Mean	0.86	164	99.6	198	20.8	627	0.6283	Moderate	P>0.05	76.5	77.2	57.9	22	135.7	46.0	5.72	Severe	3.35
	Quotient	0.09	0.44	0.37	0.92	0.41	1.53				2.61	2.66	2.99	0.52	21.9	5.83			
Al-Amarah	Mean	0.745	160	97	190	19.5	617	0.6190	Moderate	P>0.05	70.1	72.2	565	20	129	45.7	5.61	Severe	3.27
	Quotient	0.098	0.45	0.34	0.86	0.37	1.38				2.47	2.48	2.85	0.47	20.4	5.33			
Ar Ramadi	Mean	9	8	17	25	4	81	0.230	Low	P<0.05	1.8	1.9	2.8	2.4	1.1	1.8	1.122	Low	0.100
	Quotient	0.302	0.02	0.07	0.13	0.01	0.21				0.07	0.07	0.15	0.07	0.21	0.27			
As Samawah	Mean	0.653	143	87.5	181	17.8	605	0.570	Moderate	P>0.05	67.0	65.1	556	19	123.5	34.8	3.62	Low	0.980
	Quotient	0.080	0.43	0.35	0.89	0.40	1.45				2.50	2.51	2.89	0.48	20.81	5.42			
An Nasiria	Mean	0.648	142	86.2	187	17	604	0.550	Moderate	P>0.05	58.1	58.2	558	19.9	120.2	34.6	4.51	Low	0.9600
	Quotient	0.078	0.42	0.33	0.88	0.39	1.43				2.37	2.38	2.79	0.47	20.63	5.32			
Al-Hammar Marsh	Mean	0.639	140	84.1	176	17.6	608	0.530	Moderate	P>0.05	57.1	58.0	546	19.2	119.9	40.0	4.41	Low	0.7900
	Quotient	0.069	0.42	0.31	0.88	0.39	1.40				2.36	2.37	2.69	0.45	2.42	5.21			
Basrah	Mean	0.697	153	88.5	187	19.5	616	0.527	Moderate	P>0.05	66.5	66.8	567	20.9	124.5	35.7	4.67	Low	0.2100
	Quotient	0.089	0.44	0.37	0.91	0.40	1.53				2.61	2.65	2.97	0.52	21.8	5.81			

River for all the pollutants studied (P < 0.05). Correlation analyses indicated a positive correlation between the concentrations of all metals and pesticides and the level of asymmetry (r- values > 0.7).

Discussion

The results indicated that heavy metals and organic pollutants in the Baghdad and Mysan areas were linked to increased bilateral anomaly. Newman and Clements (2007) demonstrated that organisms can be influenced by different pollution levels. Different types of pollutants that accumulate in fish body tissues can directly influence metabolism and development (Heath 1995, Sindermann 1979, Newman and Clements 2007), while fish development can be affected indirectly by pollutants through their influence on prey (Newman and Clements 2007). It has been shown that microbenthic organism assemblages that are the food items of fish that bottom feed are directly affected by heavy metal and organic contamination in rivers (Stark 1998, Wright and Burgin 2009). In turn, less food leads to developmental instability in fish, which includes skeletal tissues and bone shape formation (Tacon 1992, Waagbř et al. 2005).

In fishes pollution can cause many morphological anomalies, *inter alia*, in body proportions and other osteological elements (Sindermann 1979, Pohl 1990, Lindesjoo and Thulin 1992, Sun et al. 2009); therefore, these deformities can be considered to be biomarkers of contamination. In the present study, significant variation in the value of the fluctuating asymmetry index was observed among areas with the highest and lowest levels of pollution. Analysis of variance showed that the fluctuating asymmetry values for the eight morphological characters studied differed significantly among the eight populations of *S. triostegus*. There was a significant level of contamination by different types of pollution such organic pollutants and heavy metals in areas where the fish were collected at all sites from the north to the south of Iraq (Kassim et al. 1997, Al-Lami and Al-Jaberi

2002, Al-Noor et al. 2013, Al-Obaidy et al. 2014). It is likely that pollution was responsible for the high fluctuating asymmetry values noted in these areas. Indeed, numerous studies have demonstrated that pollution was responsible for high fluctuating asymmetry values (e.g., Romanov and Kovalov 2004, Mabrouk et al. 2014, Lajus et al. 2015). In general, the toxicity of several trace metals and other chemicals has been shown to grow with increased temperature and salinity (Sogorb et al. 1988, Wright 1995, Rainbow 1997, Kwok and Leung 2005). The water temperature at Mosul was usually lower (11.9 to 20°C) (Al-Sanjari and Al-Tamimi 2009) than that at the other sites in central, west, and south Iraq (15 to 40°C) (Al-Noor et al. 2013, Hassan et al. 2014, Abbas et al. 2015). Therefore, the role of the low temperatures in increasing toxicity at the Mosul sampling site was clear from the low fluctuating asymmetry values noted there. On the other hand, because of the presence of certain salinity levels in the lower reaches of the Tigris, Euphrates, and Shatt al-Arab rivers (0.5 to 1.0%) (Kadhim 2014, Hassan et al. 2014), salinity was a factor that enhanced pollutant toxicity in these localities, and the high values of fluctuating asymmetry could have been correlated with this factor. However, it was impossible to take salinity into consideration with regard to increased toxicity at the sampling sites in central and western Iraq because of the low water salinities there (Jehad 1984, Al-Timimi and Al-Gafily 2009).

Authors like Palmer and Strobeck (2003) and Graham et al. (2010) have shown that in studies on fluctuating asymmetry, the relationship between character size and its asymmetry has important logical issues. Graham et al. (2010) indicated that asymmetry values usually increase with character size because of multiplicative errors during growth. This positive relationship was reflected in the results of the present study and in the positive relationships observed between standardized asymmetry values and *S. triostegus* size. A possible explanation of this asymmetry vale–character size relationship was increased asymmetry values with age. Juveniles have different life strategies than do adult fish, and their feeding preferences are also different from those of

adults. However, when they reach the stage at which they begin to feed on bottom organisms, they are more exposed to pollution stress, which results in increased asymmetry values (Lajus et al. 2015).

S. triostegus is widely distributed in the Tigris-Euphrates river basin (Coad 2010); therefore, early pelagic stages of the species have dispersed widely so that fish from different locations can be expected to be genetically similar. Therefore, differences in environmental conditions are the most likely explanation for the observed differences in body proportions (Booth and Schultz 1999). Differences in some head proportions of the head noted in the present study could have been linked to dietary differences (Adams et al. 2003, Blackie et al. 2003). There was a significant correlation (P < 0.001) between the upper and lower jaws and heavy metal pollution (r^2 = 0.823, P< 0.001), which could indicate changes of a morphological character in response to pollution. Similar results were obtained by Lajus et al. (2015) on the Australian estuarine smooth toadfish *Tetractenos glaber* (Fréminville). Specific variation in body part proportions is represented by directional asymmetry (Palmer 1994).

Variation in response to pollution in fish anatomical structures is evident. Michaelsen et al. (2015) showed that the effects on the eye of oil pollution are distinct, but not on paired fin length. Theses researchers suggested that asymmetry effects on eyes were lesser than that on paired fins. On the other hand, experimental studies by Allenbach (2011) have shown that eye asymmetry is more frequently linked to environmental stresses than is paired fin asymmetry. Palmer and Strobeck (1986) supported this suggestion and proposed that the characters that are functionally important are less sensitive indicators of an organism's developmental instability. In the present study, eye diameter asymmetry was high and similar to the asymmetry of the other morphological traits studied.

Generally, the present results demonstrated an association between pollution level and biological effect. The waters in the vicinity of Baghdad and the analysis of sediments showed that pollution and asymmetry levels were higher than in the waters of the other cities studied. Chemical analysis detected lower pollution levels in the waters in the vicinities of Mosul and Ramadi. Given that the other environmental conditions were moderately heterogeneous within each estuary but did not differ systematically between the two cities, the differences in asymmetry levels noted were likely linked to pollution levels.

In the present study, it was not possible to separate the effects of heavy metal and organic pollution since they were positively correlated. This differs from the results of Lajus et al. (2015). No differences in asymmetry levels were noted where spatial differences in pollution were evident in the waters in the vicinity of each city. This could have been because of local migrations of *S. triostegus* or possibly because differences in stress among sampling sites were too small.

Conflict of interest. The authors declare there were no conflicts of interest.

Ethical statement. The study was conducting according to the ethical standards.

Author's contributions. LAJ: paper concept, experimental design, data analysis, manuscript writing; MIGA: fish specimen collection, fish measurements, data analysis; JR: data analysis.

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