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Utilizing fluctuation asymmetry to assess the effects of U-238 radioactivity on the marine ecosystem around the Madras nuclear power plant, India

by

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Abstract

The monitoring of marine ecosystems is crucial due to the growing threat posed by nuclear power plants and other nuclear anthropogenic emissions. In our work, we used a straightforward and low-cost biomonitoring technique called fluctuation asymmetry (FA) to examine the variation between the left and right sides (developmental instability) of organisms' traits that were influenced by genetic and environmental variables in the early stages of ontogenesis. The specimens of fish (*Leiognathus* sp.) and crabs (*Portunus sanguinolentus*) were collected seasonally and used as bioindicators to determine the effects of Uranium-238 (U-238) radioactivity around a nuclear power plant. The obtained results revealed that FA values were not considered typical values (FA = 0) in all seasons. Moreover, FA values of *Leiognathus* sp. exhibited insignificant fluctuation for a particular characteristic through the different seasons, while a significant fluctuation occurred amongst the characteristics themselves throughout the same season. Inversely, FA values of the four characteristics in *Portunus sanguinolentus* displayed seasonal variation amongst them all. Statistically, there was a strong positive correlation (r = 0.5, $p < 0.05$) between U-238 radioactivity in the flesh of both organisms and the fluctuation asymmetry of different traits but it is not a sign that any radioactive pollution exists.

Key words: Biomonitoring, radioactivity, fluctuation asymmetry, nuclear power plant, Madras, monsoon

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1. Introduction

Nuclear power plants produce about 13.5% of the world's electricity and are the most intense thermal power source with the lowest global CO_2 emissions (Pioro & Duffey 2015). Their construction and operation had a significant effect on the marine environment; these effects can be either transitory or long-lasting. IAEA (1999) reported that, 85 PBq (1×10^{15} Bq) of solid nuclear waste were spilled directly into the marine environment from 1948 to 1993. Despite this, these nuclear man-made and natural radioactive events provide less of a risk than big disasters like the Chernobyl and Fukushima nuclear power plants disasters, which resulted in significant radioactive material emissions into the environment (Buesseler et al. 2012). Like human health, the health of the marine environment cannot be directly assessed; nevertheless, indicators of the organisms' health can be evaluated and used to determine the "health" condition of the marine ecosystem. As a result, sensitive and efficient biomonitoring techniques are required for all strategic initiatives, from categorization of discharges to contamination source management, monitoring of current circumstances and emerging trends, and evaluations of "marine ecosystem health" (Wells 1999, Salah-Tantawy et al. 2022). Biomonitoring is the use of temporal and geographical variations in a subset of biological systems and/or characteristics to detect alterations in the state of the environment.

Fluctuating asymmetry (FA) is a biomonitoring technique used to gauge the "health" of the marine ecosystem. It is regarded as a valid factor for evaluating the developmental stability (the ability of species to retain a steady state under a variety of environmental variables and pressures) of species because it reflects both genetic and environmental stressors (Mustafa et al. 2021). According to a fundamental premise of FA analysis, the development of the two sides of a bilaterally symmetrical organism is frequently impacted by identical genes; as a result, non-directional discrepancies between the sides must have environmental origins and reflect developmental accidents (Palmer 1994, Gangestad & Thornhill 1999). Typically, fluctuating bilateral asymmetry calculates the symmetry of an organism's right and left sides, so that it is symmetrically dispersed around zero.

We have studied the results of numerous studies that biomonitor the impact of radioactive substances on the health of the ecosystem using an indicator, such as fish or crab (Allenbach 2011; Bogutskaya et al. 2011; Lajus et al. 2014; Fuller et al. 2022). Furthermore, Hutchinson (1966) stated that the DNA is the most vital biochemical target of radiation due to its crucial role in cell replication and proliferation, and that unrepaired DNA damage may lead to abnormalities, genomic instability, and the death of cells. Therefore, our research aims to improve, validate, and complete those studies with seasonal data about the values and correlation between the FA of fish and crab as bioindicators and U-238 radioactivity as environmental stress, depending on the causal link between FA and the developmental instability of those marine organisms.

2. Materials and methods

2.1. Study area and collection process

The current investigation was carried out on the eastern coast of the Bay of Bengal, specifically the area surrounding the Madras Atomic Power Station (MAPS), which is located on the beaches of Mahabalipuram and Kalpakkam (Fig. 1). As a result of the climate of India, which is greatly influenced by monsoons, as well as its implications on the seasonality of rainfall, the study was conducted in three different seasons: Post-Monsoon 2020 (PoMon), Pre-Monsoon 2020 (PrMon), and Monsoon 2021 (Mon).

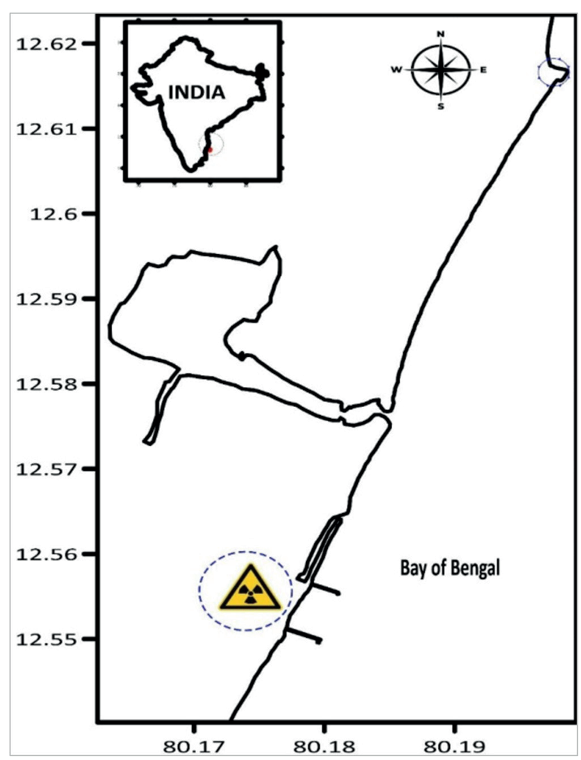

Figure 1

Map showing the sampling location around MAPS, India (AL-Sharif et al. 2023)

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A total of 60 samples of fish (*Leiognathus* sp.) and crab (*Portunus sanguinolentus*) were gathered seasonally by coastal fisher folk from the sampling station in front of MAPS. The samples were chosen and photographed using a digital camera (13-megapixel (f/1.9) camera (Exmor-RS CMOS Sensor); the images resolutions are 4128 × 3096 Pixels for morphological bilateral characters in the field beside a ruler as a size reference. The captured images were transformed with a tpsUtil32 program to convert them to tps files, and the tps files were digitized using tpsDig232 program.

Additionally, eight bilateral morphometric characters of *Leiognathus* sp were measured, and numbered from 1 to 8 as follows: Standard length, Depth of body, Lateral line, Length of eye orbital, Pre-orbital length, Pectoral fin, post-orbital length, and Pre-opercula (Fig. 2). In addition, four metrics of *Portunus sanguinolentus* were measured: length from the lateral spine to first spot, length from 1st spot to middle spot, chest length, and cheliped length (Fig. 3). All measurements were made by one operator (to avoid measurement error), the average was found by repeating measurements 3 times in different periods, and repeated measurements of 2 characters randomly chosen from each sample were analysed.

FA is appropriately measured by the equations 1 and 2 according to Yavnyuk et al. (2009):

$$AV = Xr - Xl \tag{1}$$

$$FA = \sum i|Xr \quad Xl| \quad \frac{AV}{n} \tag{2}$$

where *AV* is the average for each individual, *Xr* and *Xl* are the average character value of the right and left sides, respectively, while *n* is the number of specimens in the sample.

2.2. Assessment of U-238 radioactivity in sediments, seawater, and organisms

During the current study, twelve samples were gathered seasonally from the studied area. Three sediment samples (U SE) were collected at different seasons, cleaned from extraneous materials, air-dried, homogenized, and kept in polyethylene containers till analysis. One gram per sample was digested with a mixture of nitric, perchloric acids and hydrofluoric (3:1:1) overnight, then evaporated to near dryness to complete digestion. Following digestion, the leftover from each sample was diluted into 25 ml of ultrapure water (Millipore Direct-Q System) and filtered using 40 mm filter paper (Whatman, USA).

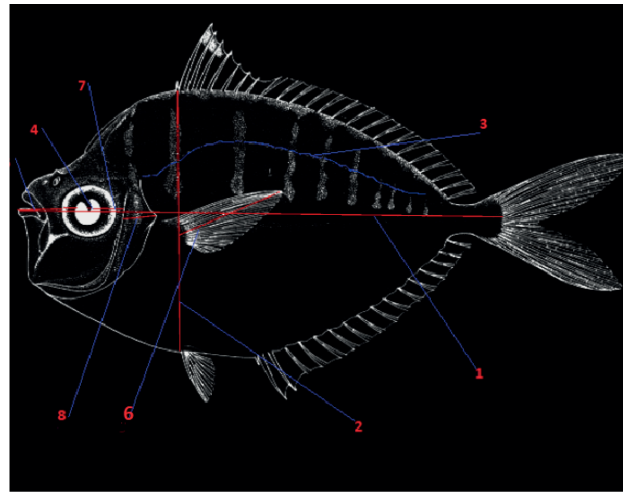

Figure 2

Bilateral morphological characters measured for FA in *Leiognathus* sp.

Moreover, three seawater samples (U SW) were collected from the sub-tidal zone in front of MAPS coast at different spots using cleaned polyethylene and glass bottles (1-liter capacity), where the pH of the samples was adjusted to 3-4.

For biological samples, we collected the six specimens from the studied area representing; *Leiognathus* sp. (U F), *Portunus sanguinolentus* (U C), identified, photographed then preserved in ethyl alcohol (70%). In our laboratory, the biological samples were washed, and the fleshy tissues were isolated, dried for 8 hrs., pulverized, and ten grams from each sample were digested with a mixture of nitric and perchloric acids (3:1) overnight, evaporated until dry, dissolved into 2 ml of HNO_3, filtered and diluted to 50 ml with double distilled water prior to analysis.

The concentrations of U-238 in sediments, seawater, and collected species were determined utilizing an Agilent 7700 series ICP-MS system available at Centre for Study on Bay of Bengal, Andhra University, Visakhapatnam, India.

Prior to being rinsed with distilled water, all equipment was washed and autoclaved for 24 hrs in a 10% HNO_3 solution. Triple digestions were performed on each sample. The resulted concentrations are expressed in parts per billion ppb. Here, we are converting the concentrations of U-238 into Bq/kg via Conversion factor (1 ppm = 12.35 Bq kg^{-1} (IAEA 1989; Joel et al. 2018).

Figure 3

Bilateral morphological characters measured for FA in *Portunus sanguinolentus*

2.3. Statistical analysis

All statistical analyses were performed using SPSS Program (V:25). The morphometric characters of *Leiognathus* sp. and *Portunus sanguinolentus*, as well as fluctuation asymmetry, were calculated. Meanwhile, Pearson's correlation analysis (*r*) carried out to illustrate the associations between bilateral morphological characters of both species, and U-238 radioactivity in sediment, seawater, and the flesh of both species ($p < 0.01$ and 0.05). All findings are represented in tables as means ± SD and visualized in figures.

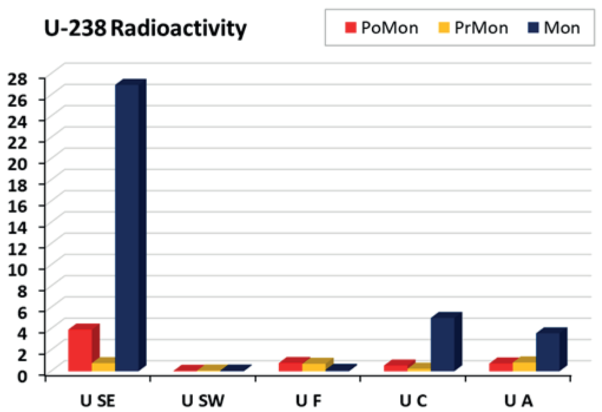

Figure 4

Radioactivity of U-238 (Bq kg^{-1}) around MAPS during different seasons

3. Results and discussion

Fig. 5 illustrated that FA values of *Leiognathus* sp showed insignificant fluctuation of the specific character through the different seasons; on the other hand, there was significant fluctuation between the characters themselves during the same season. The FA values of bilateral lateral line (character 3) recorded higher asymmetry values than other the seven characters, which recorded 0.70, 0.56 and 0.46 at PoMon, PrMon and Mon, respectively. Similarly, the concentrations of U-238 in fish showed the same seasonal trend (0.77, 0.70 and 0.17 Bq kg^{-1} at PoMon, PrMon and Mon, respectively). The seasonal patterns of U SW and U SE showed the greatest values of radioactivity (26.95 and 0.6 Bq kg^{-1}, respectively) in Mon season (Fig. 4), due to the coastal morphology

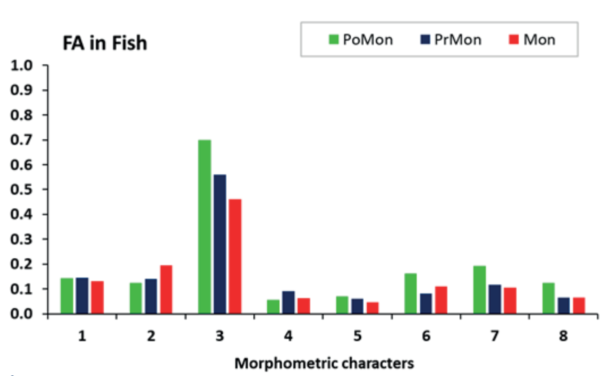

Figure 5

Bar plot depicts the fluctuating asymmetry (FA) values of eight morphometric characters of fish species (*Leiognathus* sp.) in the MAPS coast during different seasons

and processes longshore/cross-shore movement and sea current direction (Southeast /Southwest) (Aubrey 1979; Saravanan & Chandrasekar 2010; El-Taher et al. 2018; Shetty et al. 2022, AL-Sharif et al. 2023). Furthermore, the values of characters 7 and 8 reported minor values below 0.20, and they exhibited the same seasonal trend as characters 3.

The values of the standard length and body depth (characters 1 and 2) among the fish's left and right sides showed interesting findings; the FA of standard length recorded its maximum value at PrMon (0.15), while the FA of body depth had its highest value at Mon (0.19); it is rare to record an asymmetry, either mild or moderate, of these morphological features. Among the studied characters, characters 4 and 5 displayed the lowest asymmetry values (0.05 at PoMon and Mon respectively). In comparison with the previous studies, our results were inconsistent with Al-Mamry et al. (2011), whereas character 5 and 6 of the same species didn't record the highest and lowest values, respectively, and it didn't show asymmetry in character 4. Conversely, our study found negligible values of 0.05, 0.09 and 0.6 at PoMon, PrMon and Mon, respectively.

Moreover, our research contradicts Jawad (2004) and Jawad et al. (2010), who stated that adverse environmental conditions have no influence on eye diameter. Consider the efficiency of the FA technique to enhance the organism's developmental instability and the veracity of its values to improve the asymmetry between the bilateral morphological characteristics in the same individual. There is a lack of significant information that can confirm the relationship between the level and pollution type (such as radioactive pollution) and fish species' morphology; therefore, most discussions involving a variety of particular marine species are entirely based on previous research, which is already rare (Al-Mamry et al. 2011). According to Palmer & Strobeck (1986) and Palmer (1994), the FA values of our study are not considered typical values (FA = 0) but reflect the levels of environmental and genetic stressors that may have an impact on the stability of the development of the marine organism in the studied area. The current study is consistent with earlier research that demonstrated FA is a sensitive biomarker of genetic stress because fish actively develop physiologically over the first six months of life (Sheridan & Pomiankowski 1997; Barescut et al. 2009; Al-Mamry et al. 2011; Trach et al. 2022). Furthermore, the values of FA of any bilateral morphological character relies on the biology of the species to which it belongs, which varies from species to species and from characteristic to characteristic in the same individual.

Our investigation found seasonal variations in FA levels, along with U-238 radioactivity in fish and their surroundings (sediment and seawater). Such developmental consequences may occur before the level of contaminants in the water or food reaches levels high enough to induce organism morbidity (Bengtsson & Hindberg 1985). However, radiation was found to affect DNA by causing genomic malfunction (Hutchinson 1966).

Unlike *Leiognathus* sp. values, the FA values of four characteristics of *Portunus sanguinolentus* varied seasonally (Fig. 6). Characters 1 and 4 had high values in comparison with character 3, whereas characteristic 2 had light asymmetry with small values (0.02, 0.06 and 0.03). During this study, FA values of characteristic 1 (length from the lateral spine to the first spot) recorded different values with a large disparity and were the highest values between seasons and the other characters (0.07, 0.19, and 0.20 at PoMon, PrMon, and Mon, respectively). Furthermore, characteristic 1 follows the seasonal pattern of U-238 radioactivity in U SE and U C, where the highest values were recorded at Mon (26.95 and 5.02 Bq kg^{-1}, respectively), this may be linked to the behaviour and significant interactions among sediments and crab (Marijnissen et al. 2008; Suriyanarayanan et al. 2008; Cresswell et al. 2021; Siddiqui et al. 2021).

The pattern of characteristics 1 and 4 (cheliped length), observed during the current work, may be attributed to the effects of U SE and U C. This attribution was stated by Al-Mamry et al. (2011,) who demonstrated a direct association between environmental stressors and asymmetry in species. Furthermore, MAIA-LIMA (2009) stated that developmental instability in organism is induced by genetic and environmental factors that act in the initial stages of ontogenesis. During the stormy weather of MON and PrMon (Grey 1968; Grey 1975; Goswami & Mohan 2001), the examined parts (1 and 4) of crab are breakable, may be lost and renewed when hiding or any other activities through rocks hence, it is not a sign that any radioactive pollution exists (Carroll & Winn, 1989; Matheson & Gagnon 2012; Green 2004). Moderate asymmetry may occur in calm conditions like PoMon (Satpathy et al. 2019).

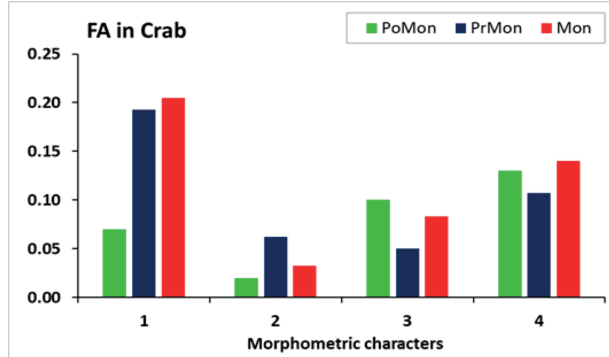

Figure 6

Bar plot depicts the fluctuating asymmetry (FA) values of four morphometric characters of crab (*Portunus sanguinolentus*) in the MAPS coast during different seasons

3.1. Correlation coefficients between FA and U-238 radioactivity.

Table 1 illustrated the association between of eight bilateral morphological characteristics of *Leiognathus* sp and U-238 radioactivity in sediment, seawater and the tissues. Minutely, there were 25 positive correlations, 18 of which were statistically significant positive correlations ($r \geq 0.5$), and 30

Table 1

Correlation relationships between U-238 radioactivity and the fluctuating asymmetry in morphometric characteristics of *Leiognathus* sp. at the studied area

Parameters	U SE	U SW	U F	trait1 F	trait2 F	trait3 F	trait4 F	trait5 F	trait6 F	trait7 F	trait8 F
U SE	1										
U SW	0.40^{NS}	1									
U F	-0.98**	-0.58*	1								
trait1 F	-0.92**	0.00^{NS}	0.81**	1							
trait2 F	0.92**	0.72**	-0.98**	-0.69*	1						
trait3 F	-0.75**	-0.91**	0.87**	0.42^{NS}	-0.94**	1					
trait4 F	-0.38^{NS}	0.69*	0.18^{NS}	0.72**	0.00^{NS}	-0.33^{NS}	1				
trait5 F	-0.81**	-0.87**	0.91**	0.50*	-0.97**	1.00**	-0.24^{NS}	1			
trait6 F	-0.03^{NS}	-0.93**	0.24^{NS}	-0.37^{NS}	-0.41^{NS}	0.69*	-0.91**	0.62*	1		
trait7 F	-0.59*	-0.98**	0.74**	0.21^{NS}	-0.85**	0.98**	-0.53*	0.95**	0.83**	1	
trait8 F	-0.40^{NS}	-1.00**	0.58*	0.00^{NS}	-0.72**	0.91**	-0.69*	0.87**	0.93**	0.98**	1

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); NS - Correlation is nonsignificant.

Table 2

Correlation relationships between U-238 radioactivity and the fluctuating asymmetry in morphometric characters of *Portunus sanguinolentus* at the studied area

Parameters	U SE	U SW	U C	trait1 C	trait2 C	trait3 C	trait4 C
U SE	1						
U SW	0.40^{NS}	1					
U C	1.00^{**}	0.45^{NS}	1				
trait1 C	0.46^{NS}	1.00^{**}	0.51^{*}	1			
trait2 C	-0.38^{NS}	0.69^{*}	-0.33^{NS}	0.64^{*}	1		
trait3 C	0.22^{NS}	-0.80^{**}	0.17^{NS}	-0.76^{**}	-0.99^{**}	1	
trait4 C	0.82^{**}	-0.19^{NS}	0.79^{**}	-0.12^{NS}	-0.84^{**}	0.74^{**}	1

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); NS - Correlation is nonsignificant.

negative correlations; 18 relationships had significant negative correlations ($r \geq -0.5$). U F has the strongest positive association with characters 1, 3, 5, 7, and 8 (r = 0.81, 0.87, 0.91, 0.74, and 0.58, respectively), while it was negative with character 2 ($r = -0.98$) and gave nothing with characters 4 and 6. With the exception of character 2, the results showed a significant negative association between U SE and characters 1, 3, 5, and 7 (r = - 0.92, - 0.75, - 0.81, and - 0.59, respectively); furthermore, characteristics 3 (- 0.91), 5 (- 0.87), 6 (- 0.93), 7 (- 0.98) and 8 ($r = -1.00$, $p = 0.01$) exhibited the same trend as U SW. Moreover, our data revealed significance positive relationship between characteristics 3, 5, 6, 7 and 8, but the characteristics 2 and 4 have a negative association with them.

Besides, there were positive correlations between characteristics 1, 4 of *Portunus sanguinolentus* and U C (r = 0.51 and 0.79, respectively), but there was no statistical association with characteristics 2 and 3. Furthermore, characteristic 1 showed strong positive correlation with U SW ($r = 1.00$, $p = 0.01$), while characteristic 3 has the negative correlation with U SW ($r = -0.80$) as shown in Table 2. These relationships are consistent with our FA findings and are supported by similar previous works (Carroll & Winn 1989; Matheson & Gagnon 2012; Green 2004). However, it does not indicate the presence of radioactive pollution in the study area.

4. Conclusion

This research used the FA technique to understand the behaviour of environmental stressors and pollutants, notably radioactive ones and the developmental instability of marine creatures. The study provides some data regarding the seasonal variation of FA and the direct relationship among specific morphological bilateral characteristics of fish and crabs and the radioactivity of U-238 in their flesh and surrounding environment.

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