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Evolution of Shannon entropy in a fish system (European seabass, *Dicentrarchus labrax*) during exposure to sodium selenite *(Na_2SeO_3)*

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Abstract: Present work shows the application of a Biological Warning System based on computer image and Shannon entropy analysis where fish´s group swim response is measured. This kind of non-invasive approach requieres the fish system to react in a quantifiable manner when exposed to perturbations. In this experiment European seabass (*Dicentrarchus labrax*) was exposed to sodium selenite (Na_2SeO_3, 10 µg/l) in order to i) test the methodology proposed by Eguiraun (doi: 10.3390/e16116133, Entropy, 2014) and ii) to quantify the effect of the Na_2SeO_3 on the fish system. Two experimental cases where performed: C_1 (control) and C_2 (Na_2SeO_3 exposure) for 7 days. Fish were video monitored daily, and fish´s group centroid trajectory was analized using Shannon Entropy (SE) in 3.5 minutes video sequences. Two sequences were recorded per day in C_1: basal (rest state) and event response (hit in the tank) while only one sequence was recorded in C_2: the event response. Results show that in C_1 the SE of the basal state (4.52) was lower than the SE of the event response (5.26), while the SE of the event response in C_2 showed a slightly lower but not significantly different value (5.10) than the control group.

Keywords: Shannon entropy; nonlinear analysis; image analysis; clustering; seafood safety; fish welfare; fish behaviour; environmental monitoring; aquaculture; biological warning systems.

1. Introduction

One of the challenges to the aquaculture industry is the lack of non-invasive, fast, easy, inexpensive methodologies to estimate the welfare of the fish and/or the detection of diseased and contaminated fish groups. The entrance points for undesirable agents, which affect fish welfare by infecting or contaminating fish groups, are usually the prey/feed, veterinary treatments (for farmed fish) and the environment (for both farmed and wild fish). Monitoring of the critical points of the production in aquaculture production would allow the early identification of the contamination and isolating or destroying the tainted fish. It has been, already, proposed the application of biological warning systems to monitor fish production, in a non invasive manner, in aquaculture [1] and developed a tool for this purpose in [2].

The purpose of this work is to test the above-mentioned tool in an experimental setting where the fish where exposed to sodium selenite (Na_2SeO_3). There exist two main reason to select this compound. First, it has been proposed as capable of neutralizing the toxic effect of a envorinmental contaminant considered a risk factor in fish consumption, named methylmercury (CH_3ClHg). Second, it shows a high antioxidant effect [3–9].

Na_2SeO_3 is a common form of selenium used as a supplement in foods and feed. Although its has been used in previous experimental works [10], its use and optimal dose is still subject to some controversy [11]. For example in humans, there is a very narrow range between the optimal daily ingestion of selenium and a toxic dose which is dependant on the selenium compound [11].

Regarding this work, it is expected that the exposure to Na_2SeO_3 should not have a negative effect in fish and indeed would protect the fish from future contaminant exposure, i.e. CH_3ClHg exposure [10]. Thus, two experimental cases should not reflect significant variations in their SE.

2. Material and Methods.

2.1. Biological Material and Water/environmental conditions

Fish were European seabass (*Dicentrarchus labrax*, 4±2 g, 8±1 cm) generously provided by Grupo Tinamenor (Cantabria, Spain). They were transported to our lab in their own seawater with constant aeration and acclimated for 1 week in 1,800 L epoxy-coated fibreglass tanks containing aerated, circulating seawater at 15 °C. During all the experimental period the fish were subject to a 12 h /12 h dark/light photoperiod and they were fed once a day INICIO Plus feed from BioMar (56% crude protein, 18% crude fat) according to manufacturers specifications for fish size, biomass and water temperature. According to its size fish were considered sexually inmature [12].

Salinity was measured prior to the beginning of the experiment by a multiparametric meter HANNA HI98192 and was 33 gr/l. The values of ammonia, nitrate, dissolved O_2, temperature and pH

were kept in non-stressful levels. Seawater was naturally sand filtered and pumped directly from the Cantabric sea in the North of the Iberian Peninsula (43°24'49.5"N 2°57'06.5"W). Additional air supply difussed by stone was introduced per tank, interrupted only during the time necessary for perform the recordings in order to avoid artefacts in the images.

All experimental protocols and procedures conducted in the present experiment were carried out under the approbation of The Ethical Committee of the University of the Basque Country UPV/EHU for Animal Welfare No. CEBA/285/2013MG.

2.2. Experimental Cases and Exposure Period

76 fish were analized in each of two experimental tanks. C_1 was the control group and C_2 was the group exposed to Na_2SeO_3. The selenium compound was Sigma-Aldrich number S5251-10G. Each of the experimental cases was formed by a tank (100 cm x 100 cm x 90 cm), which was filled up with 810 L of seawater and was placed under direct artificial white light (2 x 58 W and 5200 lm) avoiding shadows as much as possible. Same experimental conditions were replicated in both tanks.

Fish were previously acclimatized for 3 days. First experimental day, was considered a control day in both tanks and was named as day 0. After record of fish were made in day 0, fish in C_2 were exposed during next 6 days to a dosis of 10 μg of Na_2SeO_3 per liter according to Branco [10]. In order to ensure that the concentration of Na_2SeO_3 remains constant during the exposure period, the water flux was mantained closed. Thus, the water in both tanks was renewed to reduce nitrogen residuals and dirty. Water change days were day 3 and day 4 (Table 1). After water change, C_2 was exposed again. In order to minimize stress to the fish, they remained in the tanks during the changes of water. No mortality was registered.

2.3. Image acquisition, post processing, trajectory estimation and non-linear trajectory analysis

The methodology used is the same described in Eguiraun et al. [2] with the updates regarding clusters´centroid trajectory signal construction and normalization; and measurement of a rest state, added latter by same authors and described in Eguiraun et al. [13]. In short, the trajectory signal of fish´s group formed by the elements detected in each frame is built. This trajectory signal belongs to a 3.5 min videoclip of the basal or rest state and the event response of the fish. From the several algorithms tested (results not shown), Shannon entropy [14] provided the best results.

Entropy measurements are widely used and have been decisive to understand the non-linear nature of a system while providing a very powerful tool to measure the complexity of time series signals (i.e. physics [15], protein sequence analysis [16,17], telecommunications [18], physiology [19], speech recognition [20,21] and economy [22]).

Shortening, the Shannon entropy of the fish system was measured in a basal state and as a response to an event on the same normalized, by z-score, trajectory signals formed by the clusters´ centroid over the videoclip in both axis, X and Y. The basal and event responses are measured in the control group C_1 while only the event response is measured the exposed group C_2. All images where processed using the same computational parameters in despite the turbidity grade presented.

3. Results

Normalized X and Y signals had the same value of Shannon entropy, thus only results for one of them are displayed. The daily evolution of the Shannon entropy in the two experimental cases is shown in Table 1. As mentioned, day 0 was used as control of the beginning of the experiment. Thus data from this day was not taken into acount for analizing the evolution of both experimental cases. Table 1 shows the mean and standard deviation of Shannon entropy values of C_1 and C_2 from day 1 to day 6. Day 3 and day 4 (denoted with *) suffered a water change and consecuently fish were stressed. Table 2 summarizes the averaged values of Shannon entropy from day 1 to day 6.

Table 1 Results of the Shannon entropy of the trajectory of the fish cluster centroid per day and experimental case. Days denoted with (*) suffered a water change. Three basal results and the averaged measure of C_1, non-exposed case; and event responses for C_1 and C_2, Na_2SeO_3 exposed case, are shown. Day 0 was considered control day.

Day	C_1					C_2
	Basal	**Basal**	**Basal**	**Average Basal**	**Event**	**Event**
0	5.3707	5.3300	5.4184	5.3697±0.0492	5.6393	5.0132
1	4.6969	4.4265	4.6291	4.5842±0.1407	4.7242	5.1670
2	4.3904	4.0520	4.5787	4.3403±0.2669	5.0367	4.7467
3*	4.9046	4.7100	4.5365	4.7170±0.1842	6.2145	4.7868
4*	4.8874	4.8016	4.7104	4.7998±0.0885	6.0924	5.8601
5	4.6227	4.6259	4.5987	4.6158±0.0149	5.1643	5.2968
6	4.1976	4.0003	4.0387	4.0789±0.1046	4.3488	4.7703

Table 2 Exposure summary. Shannon entropy of C_1, non exposed case, and C_2, exposed case, are summarized in terms of averaged per 6 days exposure.

		Shannon Entropy
C_1	**Basal**	4.5227±0.0905
	Event	5.2635±0.7457
C_2	**Event**	5.1046±0.4365

4. Discussion

The higher SE values of the event response suggest the idea that the event response induces a higher energy dispersion of the system than the basal response. Also, attending to the low basal values of day 2 and day 6, it can be postulated that the turbidity and water change induced not only stress to the fish, but also probably made the lost of valuable information due to the image processing thresholds. Nevertheless, and regarding event response, C_1 and C_2, present a similar averaged values in terms of Shannon entropy during the exposure period and in day 0, despite turbidity and water change.

Taking togeteher these facts and given the absence of physiological and biochemical parameters that may substantiate the differences between C_1 and C_2, it can be suggested that the basal state is more

difficult to characterize due to arbitrarity and different circadian cycles of the fish. Oppositely, the fish´s response to a sudden perturbation can be a more reliable way of measuring fish activity level. Consecuently, SE values from C_1 and C_2 during the event, suggest that both cases have a nearly similar behaviour in terms of energy exchange, meaning, that exposure of Na_2SeO_3 does not affect the energy balance of the fish system.

5. Conclusions and Future Work

In conclusion, the present work describes a case study of the methodology proposed my Eguiraun et al. [2,13]. The 6-day exposure period did not seem to be a long enough period to achieve any measurable effect, which could be reflected in fish basal state, or event responses. Probably, the dose provided is not sufficient to affect fish. SE values seemed to follow some variations according the turbidity grade, probably due to the underwater visual conditions and to the fish stress originated by the lack of recirculating water and water changes. In short, and with current data in hand, it can be concluded that Na_2SeO_3 exposure does not affect fish in a quantificable manner using the proposed non invasive monitoring methodology.

Further work should be done in order to characterize a better model of the fish system with different experimental setups in order to reduce fish stress due to water change.

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Author Contributions

H.E. contributed to the design of the experiment, planned and performed the data analysis and interpretation of the results, and wrote the manuscript; I.M. designed the experiment and contributed to the interpretation of the results and drafting of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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