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Impact of different types of crude oil on embryonic neurotransmitters

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The effect of crude oil on contents of different neurotransmitters during sturgeon embryogenesis has been studied. Experiments have shown that different type of crude oils produce diverse responses of neurotransmitter systems, supporting their role as a target for developmental toxicity of crude oil.

Keywords: *Embryonic development, crude oil, neurotransmitters*

INTRODUCTION

In spite of commitment of modern hi-tech society to renewable sources of energy, the crude oil is still the major energy source and is expected to remain so for very long forthcoming time. The growth of world energy consumption is the principal driver behind massive offshore upstream investments and expansion of oil and gas transportation networks (BP Statistical Review of World Energy, 1975-2015). Alongside the coastal-based oil industry facilities (downstream sector) and natural seepage, the offshore activities and midstream oil operations contribute to massive discharge of crude oil into marine environment that is the global hot-button issue (Anderson et al., 2012). Ecosystem's response to crude oil pollution depends mainly on two factors: the type of oil and the ecological characteristics of the affected area. Complex chemical composition of crude oil determines biological and physical properties, the fate and behavior of oil in marine waters. Environmental abiotic characteristics (temperature, water chemistry and salinity, oxygen level, agricultural and urban pollutions) interfere heavily with oil toxicity making the impact on ecological networks unpredictable. The other important aspect is marine organisms' sensitivity to crude oil pollution, which varies between species, ages (developmental stage) and individual properties.

The phenotypic expressions of crude oils toxicity are multiple and include carcinogenicity, immune deficiency and high disease susceptibility, behavioral impairment, endocrine disruptions, reproduction effect, developmental malformation etc. Target specificity effect of different types of oil is a subject for debate because the underlying mechanisms of these effects still need to be elucidated (Dubansky et al., 2013; The Royal Society of Canada expert panel, 2015).

Early developmental stages are particularly vulnerable to crude oil exposure (Carls et al., 2009; Hicken et al., 2011; Hodson, 2017; Incardona, 2017; Meador et al., 2019). Embryogenesis is highly orchestrated multistage process with many players to regulate developmental progression including neurotransmitters. Monoamine neurotransmitters, besides their regulation of cognitive and behavioral functions on adult life, are involved in very complex processes of embryo pattern formation via controlling gene expression program (Sullivan et al., 2016). Interference of certain individual crude oil components with neurotransmitters' functions can trigger time- and space- improper signaling cascades resulted in acute (lethality) or delayed (disease) pathological outcomes.

Based on the said premises, the work was undertaken to study the effect of different types of crude oil on expression pattern of neurotransmitters during embryogenesis.

MATERIAL AND METHODS

The experiments were conducted on the embryos of *Acipenser stellatus*, obtained from the

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broodstock at Hylly Sturgeon hatchery. The fertilization of fish eggs was performed using the semidry techniques in small volume of solution, contained either Azeri Light oil from Chirag oilfield (light crude oils, 32º API gravity), or Neft Dashlari oil (medium crude oils, 28º API gravity) at a concentration 100 ppm. On completion of fertilization procedure (about 2-3 min) and just before the eggs become sticky, they were evenly distributed in Petri dishes and incubated in a corresponding medium until hatching under natural light/dark cycle at a room temperature (24ºC). Controls were fertilized and incubated in clean water.

The identification of embryonic stages and assessment of the rate, asynchrony and defects of developmental progression were performed under stereotaxic microscope, based on chronology of advent of specific features, described by Detlaf (Детлаф и др., 1981).

At 4 developmental stages: before fertilization (bf), blastula (bl), gastrula and neurula the randomly selected embryos were sampled to measure serotonin (5-HT), dopamine (D) and noradrenaline (NA) content by indirect ELISA using polyclonal antibodies to those monoamines. The data for norepinephrine were expressed in terms of optical density. The concentrations of serotonin and dopamine were determined by interpolation of the data from standard curves, basing on the obtained values of optic densities. t-Student's test was applied to evaluate differences between the averaged values of the groups.

RESULTS

During early developmental progression each of three neurotransmitters (NTs) in control eggs has shown the same trends in the stage-dependent expression (Fig. 1). The highest level of NTs was observed in mature oocytes before fertilization (that data was treated as the starting point for dynamic assessment). Shortly after fertilization it starts dropping and progressively declines throughout blastula and gastrula until neural stage, when the level starts going up.

Noteworthy, the amplitudes of fluctuations in concentrations between stages were quite different for serotonin vs. catecholamines. In particular, it was greater for NA and D and mild for 5-HT.

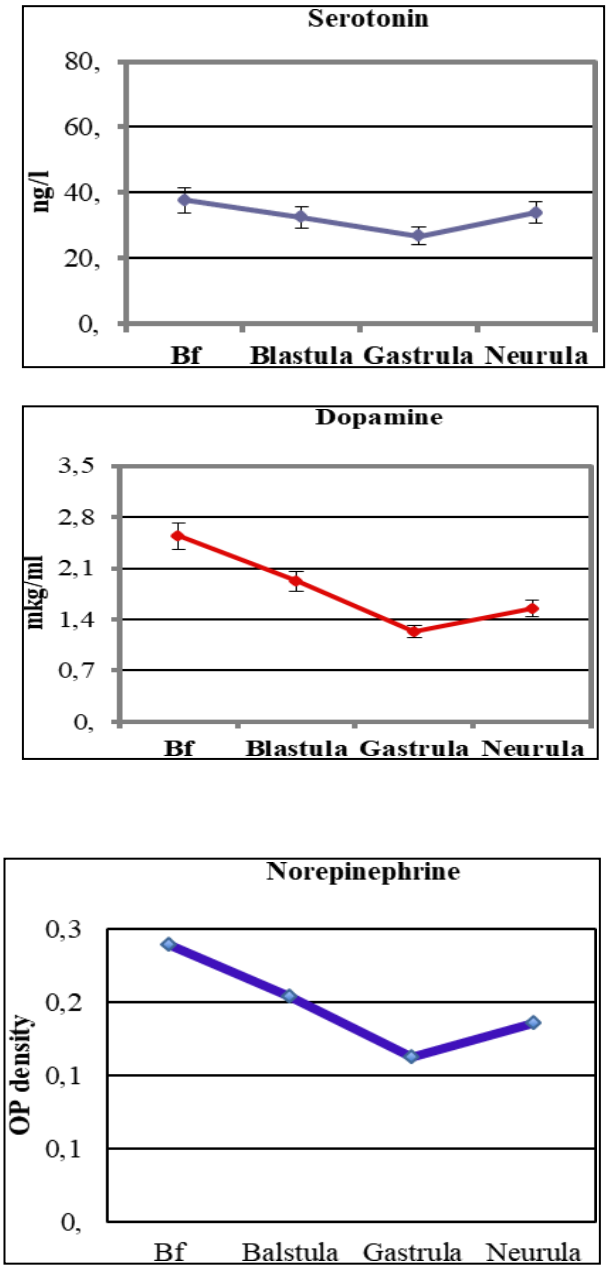

Figure 1. Changes in neurotransmitters level through early stages of embryonic development.

Exposure of fish eggs to both types of crude oil significantly downregulated of dopamine and norepinephrine and slightly depressed 5- HT in mature oocytes (Fig. 2-4). At subsequent stages of development the different types of oil produced different responses of neurotransmitter systems.

During incubation in the medium, containing the oil from the deposit Neft Dashlari, changes of

the levels of all three NTs in the fish eggs decreased markedly, though the stage-dependent fluctuations were similar to the controls up to the neurula stage, when the expected upregulation of NTs did not occur.

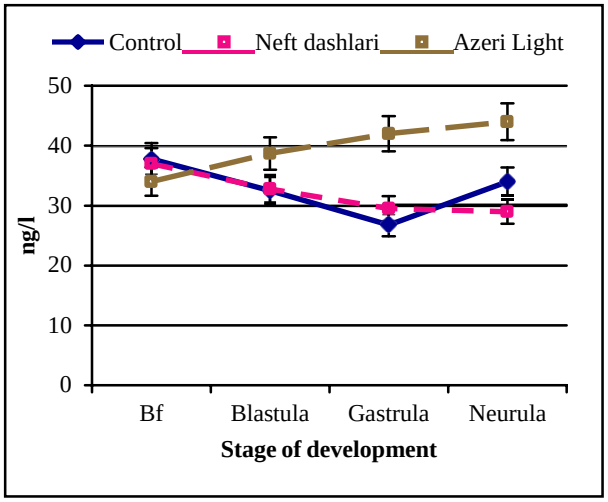

Figure 2. Changes in Serotonin level through early stages of embryonic development under different types of crude oil exposure.

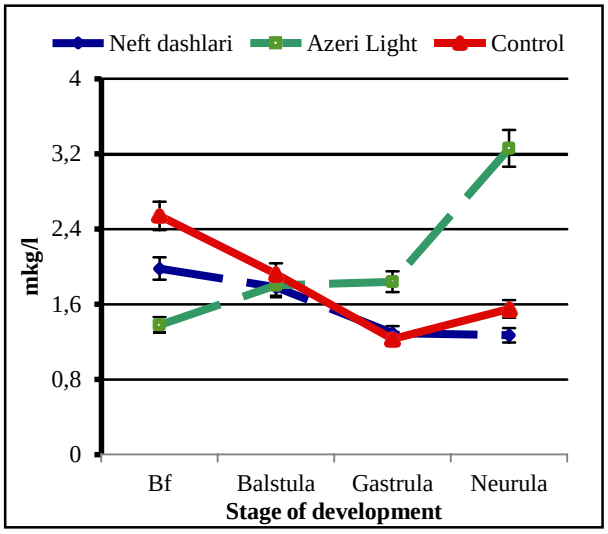

Figure 3. Changes in Dopamine level through early stages of embryonic development under different types of crude oil exposure.

Exposure to Azeri light oil caused upregulation of neurotransmitters at all stages of embryonic development. The effect was prominent on both NA via increasing content and flattering its fluctuation, and DA via bringing about twofold increase at the neurula stage in comparison with the controls ($p < 0.05$; Fig. 3).

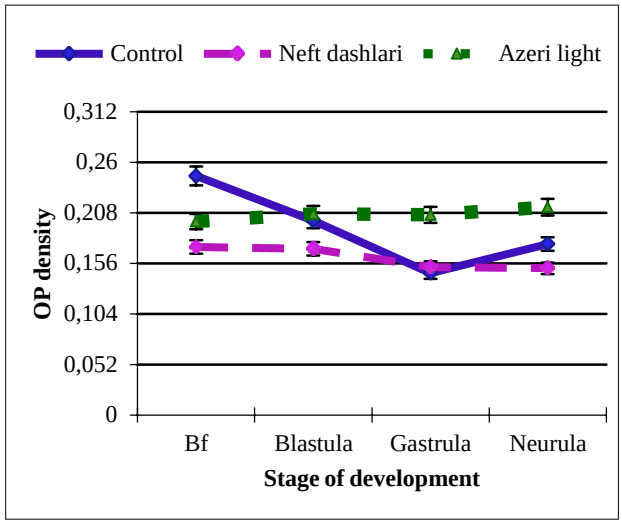

Figure 4. Changes in Norepinephrine level through early stages of embryonic development under different types of crude oil exposure

On the whole, exposure to different types of oil resulted in changes of neurotransmitter's expression pattern: Azeri light oil had stimulating effect, whereas the Neft Dashlari oil decreased neurotransmitters content. Besides, the higher hatching rate and best survival were documented for Azeri light oil. The frequently encountered abnormalities in newly hatched larva (head and spinal malformation, deviation from bilateral symmetry, yolk sac deformation, body shortening etc.) were very common for embryo toxic effects of both crude oils.

DISCUSSION

Crude oil is the naturally occurring fossil fuel and very common pollutant in marine environment due to its natural seepage and anthropogenic spills (Anderson et al., 2012). In spite of extremely complex chemical compositions, the crude oils mainly contain the same 4 major classes of compounds: Saturates, Aromatics, Resins and Asphaltenes (acronym SARA) and very small concentration of non-hydrocarbon components like heavy metals, sulphur etc. (The Royal Society of Canada expert panel, 2015). The different pro-

portions between classes and the individual constituents determine all spectrum of the physical and chemical properties, the environmental behavior and toxicity risks of crude oils.

According to scale for gravity of American Petroleum Institute (API), there are 3 types of crude oil: light (°API>31.1; medium (31.1>°API>22.3) and heavy (°API<22.3). API roughly indicates the susceptibility of crude oil to different weathering processes, affecting its behavior, fate and chemical compositions of residues after leak into the aquatic environment.

The light type of oil contains predominantly saturated hydrocarbons and is rich in low weight aromatic; other families of hydrocarbons are present in rather low concentrations. From medium to heavy oils the abundance of resins and asphaltenes prominently increased. Commonly, the saturates are considered as the least toxic and easily biodegradable compounds; the low weight aromatics (especially, benzene, toluene, ethylbenzene, xylene - BTEX acronym) are treated to be the most toxic, water soluble components which are responsible for acute toxicity/lethality. The polyaromatic hydrocarbons (PAH), resins and asphaltenes are progressively less soluble, more polar, and typically are resistant to biodegradation and have tendency to precipitate out. Their environmental persistence contributes to sub-lethal/chronic toxicity (The Royal Society of Canada expert panel, 2015).

There are no specific signs and/or target organs, which are attributed to crude oil effects at adult life stage, however multiple manifestations of toxicity cannot be treated simply as a sum (combination) of individual compound-specific effects.

It is postulated, that the lipophilic components of crude oils and/or products of their metabolism via interaction with receptors or other responsive elements, affect the intracellular signaling pathways resulting in numerous pathological (pathophysiological) outcomes, especially if these changes occur during the early stages of embryonic development (Carls et al., 2009; Hicken et al., 2011; Hodson, 2017; Incardona, 2017; Meador et al., 2019; The Royal Society of Canada expert panel, 2015).

In our experiments changes on embryo neurotransmitters' patterns under crude oil exposure have been detected.

The persistence of neurotransmitters in mature oocytes and at all stages of embryogenesis suggested their participation in key developmental processes: cell movement, proliferation, fate determination and tissue differentiation (Sullivan et al., 2016; Thomas et al., 1995; Yavarone et al., 1993).

First, they may be responsible for cell-cell or cell-matrix communications, serving as morphogen or gradient shaping signal for other morphogens. Crude oil-induced changes in neurotransmitters expression reflect changes in their concentration gradients, which is crucial for proper spatio-temporal sequencing of developmental events. This supposed to be underlying mechanism of embryo lethality, malformation and adult life pathology, depending on the stage to be affected.

Noteworthy, that the most typical expression of developmental toxicity of crude oil is the craniofacial, body axis and cardia malformations. The same effects were described under pharmacological inhibition of serotonin and dopamine activities during certain stages of embryogenesis. There is a great deal of research that confirms the participation of serotonin and dopamine in morphogenesis of craniofacial structures (Greene et al., 2018). Other studies indicated the essential role for serotonin and norepinephrine in myocardial (pacemaker and conduction system) development (Thomas et al., 1995; Yavarone et al., 1993).

The more recent researches have shown that serotonin play a pivotal role in left-right pattering (lateralization of tissue and organs): its accumulation on right side of embryo epigenetically represses (through the histone deacetylase binding partner Mad3) transformative factor *nodal* on that side with triggering the laterality pathway on the left (Fukumoto et al., 2005). These data suggest that changes in gradients of serotonin and catecholamine neurotransmitters underlie the malformation development under to exposure crude oil.

Neurotransmitters are fundamentally involved in all steps of development of nervous system; so many pathological conditions (cognitive and behavioral disturbances) may arise from the impairment of neurotransmitter-mediated neurogenesis under early developmental exposure to the crude oil.

Besides the obvious appearance of crude oils-induced toxicity, there is another important aspect to mention. The intrusions of crude oil components or its metabolites into extra- and intra-cellular microenvironment, interfere with the embryo's developmental program, may also alter epigenetic landscape, especially during erasure of DNA methylation and re-programming normally occurring in mid-blastula stage (Mhanni et al., 2004; Perera et al., 2011; Reamon-Buettner et al., 2007; Szyf, 2011). Aberrant epigenome, responsible for late onset diseases (e.g. reproductive, immune, behavioral function), can be inherited across generations and potentially affects reproduction and survival success, resulting in decrease population density.

The last, but not the least: there are not many receptor- or pathway-based data to explain the crude oil toxicity. That is why many authors suggested that commonly observed toxic effect of crude oils should be considered as the non-specific physiological response. In our experiments different types of crude oil produced diverse response of neurotransmitters systems during embryogenesis, supporting the idea of "chemical structure-physiological response" that is the subject for further research to be studied.

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Xam neftin müxtəlif növlərinin embriogenezdə monoaminlərin dinamikasina təsiri

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Tədqiqatlarda uzunburunun (*Acipenser stellatus*) embrional inkişaf dövründə neyrotrasmitterlərin dinamikası öyrənilmişdir. Aşkar olunub ki, xam neftin növündən asılı olaraq rüşeymdə monoaminlərin qradienti dəyişir və bu dəyişiklər neftin toksiki təsirinin əsasında dura bilər.

Açar sözlər: *Embrional inkişaf, xam neft, neyrotransmitterlər*

Влияние различных типов нефти на уровень нейротрансмиттеров в эмбриогенезе

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Изучено влияние различных типов нефти на динамику экспрессии нейротрансмиттеров в эмбриогенезе севрюги *Acipenser stellatus*. Предполагается, что изменение их концентрационного градиента может лежать в основе токсического действия нефти

Ключевые слова: *Эмбриональное развитие, сырая нефть, нейротрансмиттеры*