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# The Action of Nitrogen and Phosphorus on Fertility of Invertebrate Animals (*Drosophila melanogaster*)

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**Abstract:** Pollution of surface waters with nitrates and phosphates presents a serious ecologic problem. The toxicants may provoke numerous side effects to aquatic ecosystems. This article was intended to study the action of nitrates and phosphates on reproduction rate of *Drosophila melanogaster*. In total, over 2000 of fruitflies cultivated under various concentrations of nitrates and phosphates were analyzed. It was found that the effect of toxicants depended on their concentration as well as on sex of fruitflies and type of generation ($F_1$ or $F_2$).

**Key words:** Nitrate · Phosphate · Fertility · *Drosophila*

## INTRODUCTION

Pollution of surface waters with nitrates and phosphates presents a serious ecologic problem all over the World [1-3]. This problem concerns both freshwater and costal marine systems [4]. The sources of the pollutants may be of artificial or natural origin like animal manure [5]. The toxicants may provoke numerous side effects to aquatic ecosystems including killing of both invertebrates and fishes that is probably the most dramatic manifestation of hypoxia (or anoxia) in eutrophic and hypereutrophic aquatic ecosystems with low water turnover rates [6]. At an organism level, effects of the pollutants may encompass such disruptions as teratogenic and genotoxic effects, immunosuppression and other immune-system impairments as well as sex-ratio changes and altered reproductive parameters [7]. The latter may also have evolution impact. In this connection, investigation of the effects of toxicants on reproduction of animals and other organisms seems quite important.

This article was intended to study the action of nitrates and phosphates on reproduction rate of model animal object, fruitfly *Drosophila melanogaster*.

## MATERIALS AND METHODS

*Drosophila melanogaster*, a wild type, was obtained from Department of Genetics, Moscow State University, Moscow. The invertebrate animals were cultivated in 20 ml cylindrical glass tubes with 5 ml of nutrient medium. The latter contained (per 1 l of medium): yeasts - 60 g, manna-croup - 40 g, agar - 10 g, propionic acid - 5 ml, bananas - 100 g, water - 1 l. Each tube contained 3 males and 2 females. As toxicants, the following substances were taken: phosphate salt - $Na_3PO_4$ (2 mg/L and 20 mg/l, 1 and 10 maximal allowable concentrations (MAC), respectively) and nitrate salt - $NaNO_3$ (40 and 400 mg/l, 1 and 10 MAC, respectively). The toxicants were added to growth medium while its preparation. Fertility rate as well as female to male ratio were assessed in $F_1$ and $F_2$. $F_2$ animals were cultivated on the same growth medium. Control was common for both toxicants). In total, over 2000 of fruitflies were analyzed.

## RESULTS AND DISCUSSION

*The action of nitrate nitrogen*. We obtained the following results for $F_1$ generation of *D. melanogaster*. Increase in total amount of offspring were detected after

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addition of 1 and 10 MAC of the compound in comparison with control: 464 fruitflies and 463 fruitflies, respectively (in control - 369). Addition of the toxicant at both concentrations reduced female to male ratio (at that, the effect was dose-dependent): 1 MAC - 1.36, 1 MAC - 1.17 (in control - 1. 43. Concerning $F_2$ generation of *D. melanogaster* we found that nitrate nitrogen provoked increase in fertility only at 10 MAC (213 fruitflies were obtained); 1 MAC - 92 fruitflies, control - 95 fruitflies. However, reduction of female to male ration was detected only at 1 MAC (1.19) vs. 1.37 and 1.32 at 10 MAC and control, respectively.

*The action of sodium phosphate.* As in the case with nitrate nitrogen, in $F_1$ animals we detected increase in total fertility at both concentrations - 424 fruitflies for 1 MAC and 404 fruitflies for 10 MAC. However, reduction of female to male ratio was detected only at 10 MAC of the compound (1.24) vs. 1.47 for 1 MAC. Contrary to nitrate nitrogen, sodium phosphate provoked 2-fold increase in general fertility in $F_2$ fruitflies (229 fruitflies) at 1 MAC. There was insignificant increase in total fertility at 10 MAC (102 fruitflies). However, at both concentrations we detected a dose-dependent reduction of female to male ratio: 0.97 at 1 MAC and 0.9 at 10 MAC.

*Changes in sex ratios in D. melanogaster.* Changes in sex ratios in *D. melanogaster* are presented in Table 1. It is clear from the data that females and males were differently influenced by the toxicants. Moreover, the effects were dependent on concentration and a number of generation. Lets analyze the data. In two cases (1 MAC, $NaNO_3$ and 10 MAC, $Na_3PO_4$) we detected reduction of fertility; at that, it is important to note that the changes were detected only in females of $F_2$. In any other cases, addition of nitrates and phosphates to growth medium resulted in increase of females and males of fruitflies. Under the action of nitrate nitrogen, trends in sex ratio dependent on the type of generation. For example, in the first generation both in females and males we detected more evident stimulation of fertility at 1 MAC while in the second generation, the situation was opposite - also, both in females and males, more increased fertility was detected at 10 MAC. Under the action of sodium phosphate, in females of the first and second generations, more evident increase in fertility was detected at 1 MAC. In males of the first generation, more evident increase in fertility we detected at 10 MAC while in the second generation - at 1 MAC. It should be noted here that females of the first generation reacted to toxicants in a similar way while males - in a different way (depending on a type and dose of toxicant). Alternatively, both females and males of the second generation differently reacted to both toxicants.

Lets consider the possible explanations for the detected phenomena. Fruitflies like any other organisms need nitrogen and phosphorus to build their macromolecules including proteins, nucleic acids and ATP [8]. General increase in fertility may be explained, in our opinion, by sufficient amount of the elements needed for metabolic processes. Previously, it was reported that phosphorus, for example, may influence growth rate in *D. melanogaster* [9]. It should be noted that maximal stimulation effects were detected in the second generation: it may be explained by the fact that flies in $F_2$ experienced more pronounced deficiency of nutrients in comparison with $F_1$. At that, males in both generations were more sensitive in comparison with females. This may be linked with different ability of males and females to incorporate exogenous nitrogen and phosphorus. However, despite the stimulation of fertility, changes in sex ratios (female to male ratio) seems a disturbant fact: in the nature, it may be dangerous to other animal populations.

## CONCLUSION AND FUTURE DIRECTIONS

Our study showed that additional nitrogen and phosphorus may increase fertility rate both in males and females. The effect of fertility stimulation depended both on concentration of the elements and type of organism's generation. In the future, it would be interesting to

Table 1: Changes in sex ratios in *D. melanogaster* after additions of toxicants. Negative values mean reduction in comparison with control values

| | | Toxicant | | | |
|---|---|---|---|---|---|
| | | $NaNO_3$ | | $Na_3PO_4$ | |
| Generation | Dose | Female | Male | Female | Male |
| $F_1$ | 1 MAC | 23 % | 29 % | 17 % | 13 % |
| | 10 MAC | 0 % | 22 % | 3 % | 18 % |
| $F_2$ | 1 MAC | -8 % | 2 % | 109 % | 182 % |
| | 10 MAC | 127 % | 119 % | -13 % | 27 % |

investigate the combined action of the elements since some data indicate that nitrogen may influence availability of phosphorus [10]. Besides, it would be interesting to study the influence of these elements on lifespan of fruitfly males and females. However, it needs a new research.

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