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Physiological and Reproductive Disorders of Baltic Amphipods *Gmelinoides fasciatus* Exposed to 4-tert-Octylphenol

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Abstract—Alkylphenols, which are industrial chemicals, are xenoestrogens; however, their sublethal effects on aquatic animals are poorly studied. The aim of this work is to study possible metabolic and reproductive disorders in amphipods using the example of *Gmelinoides fasciatus* from the Gulf of Finland (Baltic Sea) after experimental exposure to 4-tert-octylphenol (4t-OP) from the group of alkylphenols. The mortality of *G. fasciatus* amphipods in 4t-OP concentration ranges from 0.5 to 1000 μg/L after 96 h and 7 days increases with increasing concentration. At 4t-OP concentrations of 0.5 and 5 μg/L, the mortality of *G. fasciatus* has not been observed. At a concentration of 20 μg/L, after 7 days, the mortality of crustaceans is 20%; at a concentration of 100 μg/L, it is 60%. Exposure to sublethal concentrations of 4t-OP (0.5 μg/L) resulted in a decrease in sexual activity of these amphipods after 1 week: only 25% of amphipods retain precopulatory pairs versus 100% in control. Under chronic exposure (28 days) to the lowest concentration, males show signs of demasculinization (a decrease in the width of the gnatopods and an increase in the depth of the coxal plates); females show a decrease in the overall fecundity and irreversible disturbances in the state of embryos (a high proportion (>50%) of embryos stop their development at different stages of organogenesis). The study shows significant functional disorders of metabolic processes and reproduction in crustaceans even when exposed to nonlethal concentrations of octylphenol. These results confirm the high toxicity of this industrial pollutant, the presence of which in the aquatic environment can lead to irreversible changes in the biota.

Keywords: amphipods, rate of oxygen consumption, reproduction, state of embryos, morphological structures, gnatopods, alkylphenols, xenoestrogens, Gulf of Finland

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INTRODUCTION

Environmental pollution with hazardous substances is an acute problem of aquatic ecosystems in northwestern Russia and Europe, including the eastern part of the Gulf of Finland and the Baltic Sea (Kuprijanov et al., 2021). Compounds that behave like endogenous estrogens, called environmental estrogens or xenoestrogens, have received the most attention in recent decades. Xenoestrogens may exert their effects by binding to the estrogen receptor (Nimrod and Benson, 1996). These substances include some pharmaceuticals (e.g., 17α-ethinyl estradiol), pesticides (e.g., lindane and endosulfan), and industrial (alkylphenols, bisphenol-A, 12-phthalates, polychlorinated biphenyls, organic tin compounds, etc.) chemicals.

Alkylated phenols are a group of nonionic persistent surfactants commonly found in wastewater that can accumulate in living organisms when released into fresh and marine waters. Octylphenol is found in industrial wastewater, as it is used in the production of phenol-formaldehyde resins (98% of all sources), octylphenol ethoxylates, and ether sulfates (2%). These resins, ethoxylates, and sulfate esters are used to tackify tire rubber, as well as in the production of water-based paints and pesticides (as a dispersant). Octylphenol is also present as an impurity in industrially produced nonylphenol. The octyl group in the octylphenol structure can be linear or located in the second, third, or fourth position on the benzene ring. Of these isomers, 4-tert-octylphenol (4t-OP) is the most important for industrial purposes (Miyagawa et al., 2021). It is toxic to aquatic organisms and, at sublethal concentrations, can cause significant endocrine disruptions and developmental abnormalities in fish (Madsen et al., 2003; Kinnberg et al., 2003; Lee, 2006). However, the effects of 4t-OP and the mechanisms of its influence on aquatic animals are still poorly understood.

The present study examines the effect of alkylphenols on physiological and reproductive disorders of the amphipod *Gmelinoides fasciatus* (Stebbing, 1899) from the Gulf of Finland, Baltic Sea. Crustacean amphipods are important components of marine ecosystems, in particular the Baltic Sea. Nowadays, different amphipod species are widely used as test species for assessing environmental pollution. The amphipod *G. fasciatus* inhabits the estuarine part of the Gulf of Finland. Its biology has been well studied and its sensitivity to various types of technogenic impacts has been determined. Therefore *G. fasciatus* is considered a promising species for use in biotesting (Berezina et al., 2013, 2019). The present study is aimed at determining the effects of acute and chronic exposure to 4t-OP on amphipod survival, metabolic activity, and reproductive health. In particular, after 96 h and 7 days, the mortality of the amphipod *G. fasciatus* was determined in the range of 4t-OP concentrations from 0.5 to 1000 μg/L and the rate of oxygen consumption at concentrations of 0.5 to 20 μg/L. In a long-term experiment, we have studied the effect of nonlethal concentrations of 4t-OP on the success of the formation of precopulatory pairs, morphological characteristics of males, quality of embryos, and fertility of females.

MATERIALS AND METHODS

Experimental Design

The amphipods *Gmelinoides fasciatus* were captured in a clean habitat in the Gulf of Finland (Sestroretsk, Dubki Park) in June 2020 at a water temperature of 12°C. They were adapted to laboratory housing for a week under the following conditions: constant water temperature of 12°C, salinity 100 mg/L, pH 7.6, and photoperiod 12 : 12 h (day : night). Water for keeping the crustaceans was taken from the habitat and mixed with settled tap water in a ratio of 1 : 3. Amphipods were fed daily with a 1 : 2 mixture of animal fish food and dried algae (TetraMin$^{®}$). For the entire period in the laboratory, the amphipods were provided with shelter in the form of stones and artificial algae; aeration of the water in the aquariums was carried out using a compressor through pipettes located 5 cm from the bottom. The aquariums were covered with glass to prevent water evaporation.

Subsequently, selected from this laboratory group, the specimens were kept under similar conditions for 7 days under the influence of 4t-OP in seven concentrations (0.5, 5, 20, 100, 200, 500, and 1000 μg/L) and in a control (in the absence of this substance). Each tested group contained 40 specimens of *G. fasciatus*. Mortality rate (% of the initial number of crustaceans) was determined after 96 h and 7 days in all variants. The rate of oxygen consumption (respiration) of the amphipod *G. fasciatus* was determined after 7 days of keeping the crustaceans in three variants of 4t-OP concentrations (0.5, 5, and 20 μg/L) and in the control.

The long-term (28 days) maintenance of amphipods was carried out in the control and the variant with the lowest concentration of 4t-OP (0.5 μg/L). To do this, 120 pairs of amphipods (male + female) were selected from the control batch at the precopulatory stage and placed in each of the variants in triplicate. After exposure, the length of the body and head capsule of amphipods was measured, the fertility and condition of embryos in pregnant females were assessed, and the width of gnatopods I (right and left) and the depth of coxal plates IV of males were measured. These morphological characteristics are most variable in the case of endocrine disorders and have previously been used as one of the morphological criteria for separating normal and intersex male amphipods (Ford et al., 2004). Intersex males differed in the size of the mandibles of the first pair of pereiopods (gnatopods I), which are significantly larger in normal males than in intersex males and females. The increased depth of the coxal plates of pereiopod VI in intersex males may be greater than in normal males.

Determination of Alkylphenols in Water

In the experiments, we used chemically pure 4-tert-octylphenol 97%, $CH_3)_3CCH_2C(CH_3)_2C_6H_4OH$ (Aldrich), which was dissolved in acetone and then in water to the calculated concentration levels corresponding to the experimental options (Table 1). We used settled tap water (mineralization 0.7 g/L). Water samples (0.5 L) to determine the actual concentrations of the substance were taken from each experimental variant and the control at the beginning and end of the experiment. Water samples were kept cold at −20°C until analysis.

The preparations for the analysis of water samples (500 mL) included liquid–liquid extraction (three times 15 mL of hexane) at a neutral pH. The resulting extracts were summed up and dried over anhydrous sodium sulfate. The samples were evaporated on a rotary evaporator; the extract was transferred to a conical tube and evaporated to dryness under a stream of nitrogen. Then 50 μL of the derivatizing agent N,O-bis(trimethylyl)-trifluoroacetamide was added to the dried extracts and placed in a thermostat for 30 min at 60°C.

The quantitative content of the detected compounds was assessed using the internal standard method (2-fluoronaphthalene), which was introduced into the sample immediately before the extraction procedure. The calculation of the mass fraction for each compound was carried out by the internal standard method using the conversion factors in the alkylphenol and 2-fluoronaphthalene pair.

The analysis of alkylphenols was carried out in the SIM (Selection Ion Monitoring) target mode on a single-resolution QP 2010 chromatography mass spectrometer (GC/MS) (Shimadzu). The GC/MS recording parameters were as follows: temperature of the ion source 200°C, interface 270°C, injector 250°C, and mode with a constant flow rate of carrier gas (helium) 1.0 mL/min; ionization energy 70 eV. The analysis used a capillary column of medium polarity TR-5MS (60 × 0.25 × 0.25 mm). The volume of the injected sample was 1 μL. The column thermostat was heated according to the following program: an initial tem-

Table 1. Calculated and actual levels of 4t-OP content (μg/L) in the water samples at the beginning and at the end (7 days) of the experiment

Experimental variant	Calculated	Actual in the beginning	Actual at the end
Control	0	<0.01	<0.01
I	0.5	0.48 ± 0.01	0.48 ± 0.01
II	5	4.98 ± 0.02	4.99 ± 0.01
III	20	20.1 ± 0.02	20.0 ± 0.01
IV	100	101.1 ± 0.01	101 ± 0.01
V	200	198.9 ± 0.01	199 ± 0.01
VI	500	500.9 ± 0.01	501 ± 0.01
VII	1000	1000.1 ± 0.01	1000 ± 0.02

perature of 60°C was maintained for 1 min, then the column was heated at a rate of 15°C/min to 280°C (with a 5-min holding period). The total chromatography time was 31 min. The limit of quantification of 4t-OP was 0.01 μg/L.

Biological Indicators

The rate of oxygen consumption (OCR) of amphipods in a quiet state (normal behavior without active movement or hunting) was measured in closed 100-mL respirometers with filtered water. The OCR was calculated from the difference between oxygen levels in the experiment (with amphipods) and the control (without animals) after an exposure of 4 h. Dissolved oxygen was measured using a HANNA HI 9142 oximeter (Germany), calibrated before the start of measurements according to the standard. One measurement, which was repeated five times for each experimental variant, included from three to five adult individuals (body length from 6 to 9 mm). The wet weight of animals was determined on an analytical balance with an accuracy of 0.01 mg. Then, for comparison, the OCR was recalculated per individual (with average fresh weight of 55 mg) per hour.

Fecundity, or the number of eggs laid by a female *G. fasciatus* in nature, varies from 8 to 45, being related by a power function to the length of the female's body (Panov and Berezina, 2002). The body length of females was measured (in millimeters) and the individual fecundity of females was determined as the total number of embryos in the marsupium. Then the average fecundity values were calculated for the entire sample of females with *n* at least 20 and reduced to the same length for comparison.

Frequency of Embryonic Disorders

Seven (Pöckl, 1993) or nine (Sundelin et al., 2008) stages of egg/embryo development have been described in different amphipod species. In *G. fasciatus*, egg development lasts for 3–4 weeks at a water temperature of 12°C; we identified nine stages according to the previously presented classification (Berezina et al., 2019). A detailed description of embryonic stages is given for the amphipod *Gammarus fossarum* (Arambourou et al., 2017); it can also be used for other gammarid amphipod species, including *G. fasciatus*. Newborn crustaceans remain in the marsupium (brood pouch) of the female for several days. Embryos at stages of organogenesis, at stages IV–VIII, were carefully removed from the pouch and carefully examined for the presence of developmental defects. We used fine needles and pipettes for the dissection of the embryos.

The quality, mortality rate, and number of anomalies of *G. fasciatus* embryos were assessed using a stereoscopic microscope MBS-10 (LOMO, St. Petersburg), equipped with additional magnifying lenses. For counting and photographing, some embryos were fixed with a 0.02% glutaraldehyde solution. Photos were taken using a Nikon camera.

Four main types of easily quantifiable (Sundelin and Eriksson, 1998; Sundelin et al., 2008) aberrant amphipod embryos have been identified: (1) malformed embryos, (2) undifferentiated (when the stage of development is unclear because they have stopped developing), (3) with damaged membrane, and (4) dead embryos. Embryos are classified as malformed when they exhibit various morphological abnormalities (e.g., the presence of compound comma-formed eyes or/and reduction of rudimentary limbs and midgut that acquires an irregular club-shaped shape). Various types of membrane disruptions have also been described (Sundelin et al., 2008). The most serious disorders occur when lipids leak through the damaged membranes through the inner membrane of the embryo. Minor membrane dysfunctions can lead to disruption of osmotic regulation and accumulation of water inside the embryo, leading to edema and increased embryo size. All of the malformations listed above, except those described as "enlarged embryos" without any other abnormalities, are considered lethal (Sundelin et al., 2008).

The frequency of aberrant embryos (undifferentiated and malformed) was determined as the average

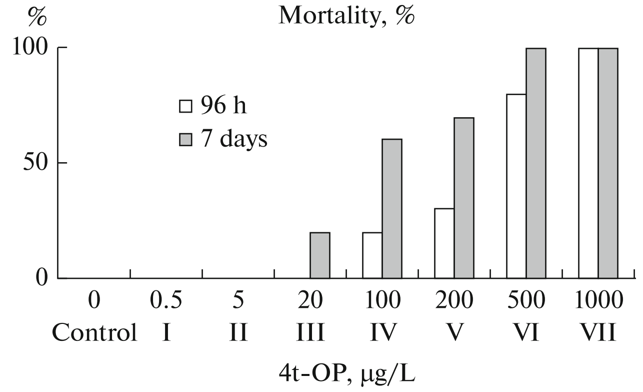

Fig. 1. Mortality rate of the crustacean amphipod *Gmelinoides fasciatus* after exposure to seven concentrations of the alkylphenol 4t-OP and in the control (in the absence of the substance).

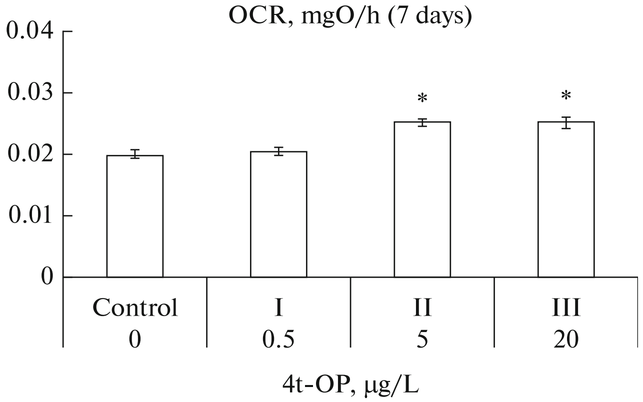

Fig. 2. Rate of oxygen consumption (mean and standard deviation) in the control and after exposure to alkylphenol 4t-OP in variants I, II, and III. Values that are statistically different from the control are marked with an asterisk.

percentage ratio of the number of aberrant embryos to the total number of embryos in the marsupium of all studied females, calculated per female of average size.

Males were examined after 28 days of being kept at a concentration of 0.5 μg/L 4t-OP and in the control. Body length, width of the head capsule, length of gnatopod I, and depth of coxal plates IV were measured under an Olympus SZX2 stereoscopic microscope with 10× eyepieces, resolution 5 μm, and magnification 20×.

Statistics

The experimental results were processed using Excel and Statistica software packages: the arithmetic mean and standard deviation were calculated; the significance of differences between options was assessed by medians using the nonparametric Kruskal–Wallis test, followed by pairwise comparison using the Mann–Whitney U test with Bonferroni correction. If the data were normally distributed (for female fecundity and male morphometric parameters), one-way analysis of variance was used, followed by pairwise comparison of samples using Student's *t*-test.

RESULTS

Alkylphenol Concentrations

The actual content of 4t-OP in the water of experimental variants (I to VII) coincided with the calculated values (Table 1). No significant differences were found between the content of the substance at the beginning and end of the experiment (7 days). In the control, the content was below the detection limits of the method; i.e., no substance was found here.

Biological Indicators

Mortality rates were high at 4t-OP concentrations over 200 μg/L (Fig. 1). The absence of mortality after 96 h and 7 days was noted at a 4t-OP concentration of 5 μg/L or lower.

The lowest tested concentration of 4t-OP (0.5 μg/L, variant I) did not have a noticeable effect on the metabolic activity of crustaceans, assessed by the rate of oxygen consumption (Fig. 2). At 4t-OP concentrations of 5 and 20 μg/L, the rate of oxygen consumption by crustaceans with an average wet body weight of 55 mg is statistically significantly higher (by 26–27%) than in the control (Kruskal–Walliss test, H = 5.33, $p = 0.021$), with a significant difference between the control and variants II and III (Mann–Whitney, $p < 0.05$).

On the 7th day of the experiment, the number of precopulatory amphipod pairs was significantly less in the variant exposed to 4t-OP (0.5 μg/L) (25% of the initial number), although their number in different variants of the experiment was equal at the beginning of the experiment (Fig. 3). In the absence of the substance (control), a noticeable decrease in the number of copulating pairs was noted only after 14 days, which is a natural process associated with molting and successful fertilization.

The embryos in the marsupium (brood pouch) of the females, examined after 28 days, were in poor condition. About 70% of the embryos were nonviable and some of the eggs were unfertilized. Most embryos stopped development at various stages of embryogenesis; they were not viable (Fig. 4).

Also, some of the dead embryos were aborted, which led to a decrease in overall fertility compared to the control (Fig. 5). At the same time, the proportion of embryos with aberrations, such as membrane dysfunction (destruction of membranes with leakage of lipids through the inner shell of the egg), was low (3–5%), both in the control and in the experiment (Fig. 5).

The morphometric parameters of male amphipods after a long exposure to alkylphenols are shown in Table 2. For most parameters, their values differed

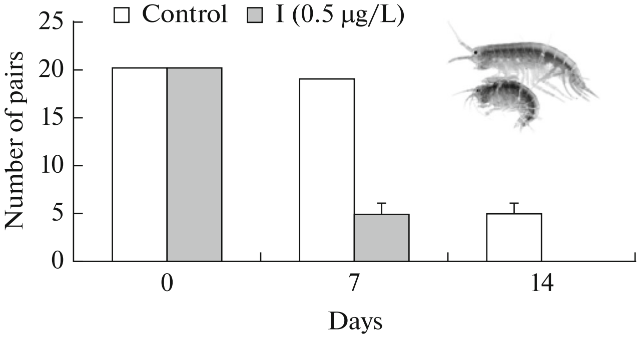

Fig. 3. Number of pairs of crustacean amphipods *Gmelinoides fasciatus* (mean and standard deviation) in the control and after exposure to 0.5 µg/L of alkylphenol 4t-OP (variant I).

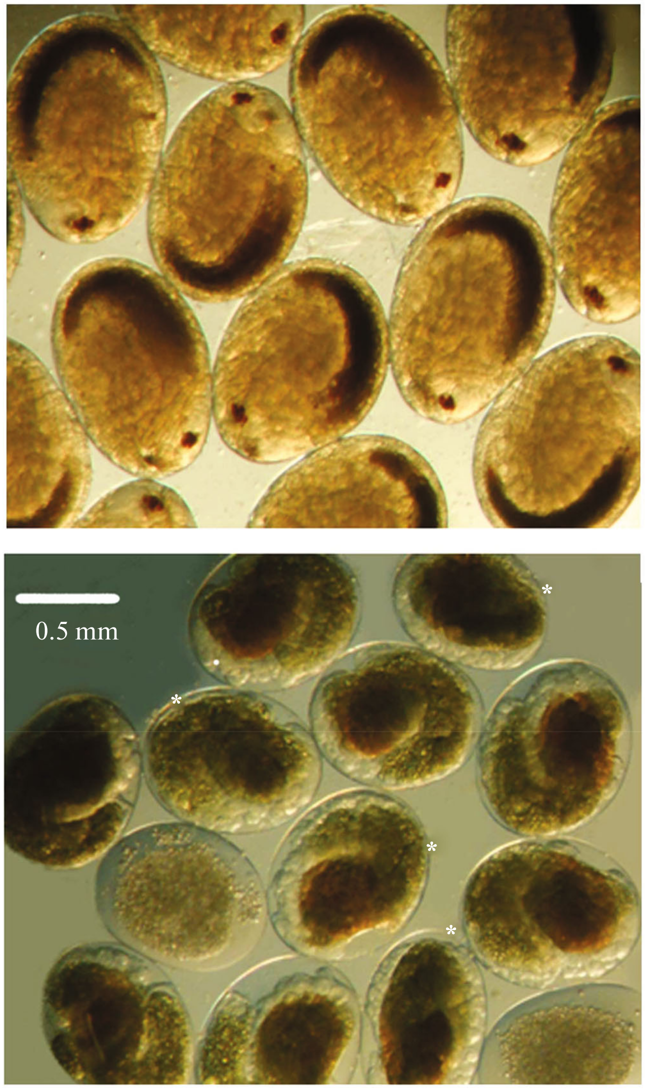

Fig. 4. Photos of amphipod *Gmelinoides fasciatus* embryos: normal (top photo) and with developmental aberrations (bottom photo marked with asterisks).

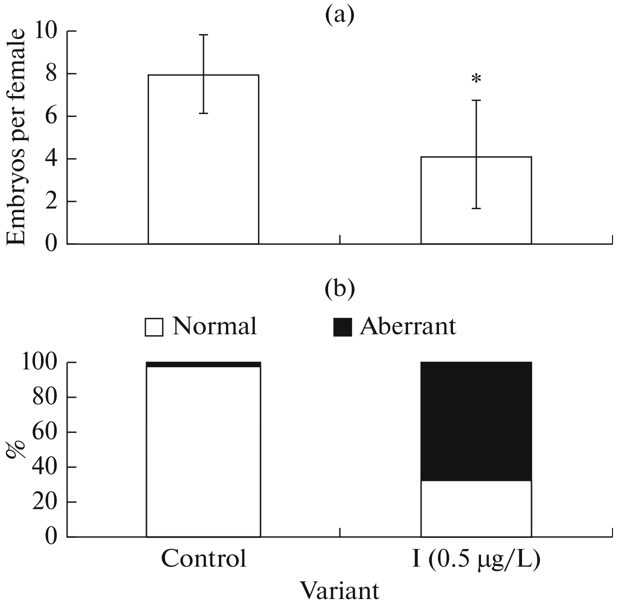

Fig. 5. Indicators of fertility and quality of embryos of the amphipod *Gmelinoides fasciatus* in the control and after 28 days of exposure to alkylphenol 4t-OP at a concentration of 0.5 µg/L (variant I). (a) Average fecundity value (and standard deviation); differences are significant according to $F_{(1,\ 28)} = 22.56$, $p < 0.0001$, t stat = 4.75 ($<t$ crit = 1.71), $p < 0.0001$). (b) Proportion of normal and malformed embryos.

between the control and the experiment (0.5 µg/L 4t-OP) at $p < 0.05$. At the same time, the males of both variants did not differ significantly in body length. At the same time, the length of gnatopod I of males of the experimental variant is approximately 20% less, and the depth of coxal plate IV is significantly greater than in the control (Fig. 6, Table 2).

DISCUSSION

Alkylphenols from the group of nonionic surfactants are industrial pollutants commonly found in wastewater and often are not very degradable in the environment. They are prone to degradation into xenoestrogenic metabolites, which contaminate various environments (including water and sediments), and can cause endocrine disruption in aquatic animals due to their estrogen-like effects (Soto et al., 1991; Gray et al., 1999). The toxic effect of octylphenols is associated with their ability to mimic natural hormones and disrupt the functioning of the endocrine system of living organisms (Soares et al., 2014). Among octylphenols, 4-tert-octylphenol (4t-OP) has the highest estrogenic activity (Sheikh et al., 2017).

In our study, 4t-OP at a concentration of 5 µg/L led to significant changes in the energy metabolism of amphipods and at a concentration of 0.5 µg/L, to reproductive disorders (such as a decrease in fertility and quality of embryos) and disturbances in sexual behavior (the breakup of precopulatory pairs). Concentrations of 20 µg/L and higher resulted in the death of the studied amphipods. In other cases, an adverse effect of octylphenols on the mammary glands of marine animals was found at concentrations of 6.1 µg/L and higher; these disorders were expressed in the proliferation of tumor cells of the gland (Soto et al., 1995). It has been shown that the formation of byssal threads by the Gray mussel (*Crenomytilus grayanus* (Dunker, 1853)) is inhibited by 4t-OP at a concentration of 1 µg/L in seawater (Kondakova and Chernyaev, 2018). Octylphenol is accepted to have adverse effects on aquatic animals at concentrations exceeding 6 µg/L (*Environmental Risk*, 2005). Such levels have been observed in natural reservoirs. For example, the measured concentration of octylphenol reached 13 µg/L in the waters of the Tisa River estuary (Blackburn and Waldock, 1995). There are little data on the content of these substances in natural water bodies, although it is known that octylphenols are determined not only in water, but accumulate also in biota and bottom sediments (Staniszewska et al., 2016; Zaitseva and Medvedeva, 2019). The levels of octylphenol and ethoxylates determined in the sediment samples collected in 2018 from five locations in the eastern part of the Gulf of Finland were low, below the detection limit of 3 µg/kg dry matter. In other areas of the Baltic Sea, octylphenol concentrations in sediments reached 100 µg/kg dry matter weight (HELCOM, 2019). Safe exposure levels (SELs) for 4t-OP in water and biota have not been determined, but the estimated SELs for octylphenols in sediments calculated by the Baltic Marine Environment Protection Commission are 17 µg/kg dry matter weight (HELCOM, 2019). However, this value requires experimental verification.

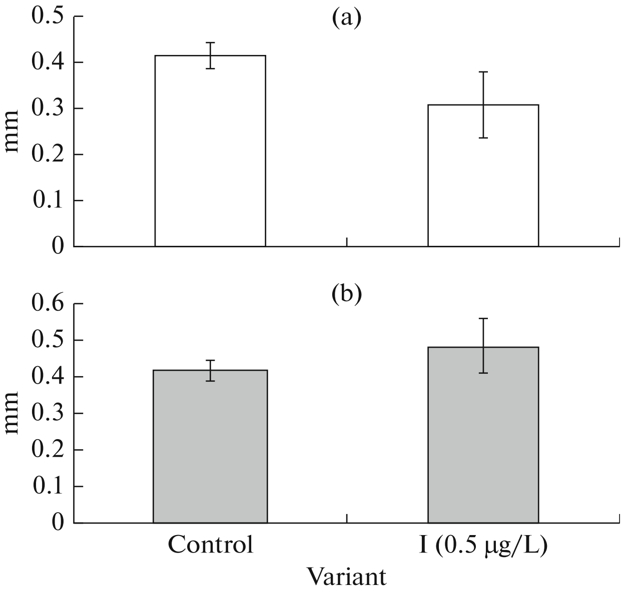

Fig. 6. Average values (and standard deviations) of the length of the right gnatopod I (a) and the depth of the coxal plate IV (b) of male *Gmelinoides fasciatus* in the control and experiment (variant I). Statistical indicators are the same as in Table 2.

Threshold levels for 4t-OP in water, which serve as a kind of benchmark for a "good surface water quality," have not yet been determined. This study showed that concentrations of about 0.5 µg/L cause endocrine-type reactions, while the death of crustaceans occurs at 40 times higher concentrations in water. Apparently, 4t-OP at concentrations of 0.5 µg/L and, especially, higher should be considered potentially dangerous for organisms in the Baltic Sea and other water bodies. Exposure to octylphenol at this concentration led to an increase in the expenditure on energy metabolism, a decrease in the success of egg fertilization (due to the antiandrogenic effect in males), and a deterioration in the quality and the number of embryos. All

Table 2. Statistics for six indicators measured in male amphipod *Gmelinoides fasciatus* in the control (C) and experimental (E) variants. Designations: BL, body length, mm; HL, head length, mm; GRL, length of the right gnatopod I, mm; GLL, length of the left gnatopod, mm; CoxD, depth of the coxal plate IV, mm; CoxW, width of the coxal plate IV, mm. The *p* values for the statistically significant differences between the two variants are highlighted in bold

	BL		HL		GRL		GLL		CoxD		CoxW	
	C	E	C	E	C	E	C	E	C	E	C	E
Mean	7.67	7.09	0.41	0.31	0.42	0.33	0.74	0.67	0.42	0.45	1.02	1.00
StE	0.14	0.25	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.01
Median	7.54	7.38	0.42	0.33	0.42	0.36	0.71	0.66	0.42	0.44	1.02	1.01
StD	0.64	1.15	0.03	0.10	0.03	0.10	0.08	0.09	0.04	0.07	0.08	0.05
Min	6.46	4.77	0.38	0.16	0.35	0.20	0.59	0.52	0.35	0.35	0.89	0.89
Max	8.92	8.86	0.47	0.46	0.47	0.47	0.87	0.82	0.47	0.56	1.11	1.08
n	21	21	21	21	21	21	21	21	12	12	12	12
t Stat	1.82		2.83		4.83		3.95		–3.54		0.06	
p	0.0841		0.0104		0.0001		0.0008		0.0046		0.9514	

these disturbances resulted in low fecundity of the population, which will subsequently lead to a decrease in the number of the species and a possible reduction in biodiversity. Therefore, current screening and monitoring methodologies for the biological effects of contaminants should consider 4t-OP a hazardous substance with a high environmental risk that may cause irreversible damage to aquatic animals.

Amphipods of various species (including *G. fasciatus*) have a precopulatory stage in the pre-molting period that precedes oviposition. During this stage, male amphipods hold mature females with gnatopods by grasping the first sternite region of the carapace, thereby increasing the possibility of egg fertilization. The reduced size of the gnatopods of *G. fasciatus* males associated with exposure to 4t-OP may reduce the chances of "controlling" females. This is indirectly confirmed by the low proportion of mating individuals after 7 days of exposure, as well as the presence of unfertilized eggs in females.

Many works are devoted to the study of reproductive disorders of amphipods of various species under the influence of water pollution both in nature and in experiments (Sundelin and Eriksson 1998; Mann and Hyne 2008; Camus and Olsen, 2008; Arambourou et al., 2017; Berezina et al., 2019). Nowadays, the possibility of using a new biomarker, such as the "frequency of malformed embryos" to assess the unfavorable state of the environment is being considered (*HELCOM*, 2023). For example, in the Gulf of Bothnia (northern Baltic Sea), a correlation was observed between the distance to an industrial plant and the frequency of detection of aberrant amphipod embryos (Sundelin et al., 2008; Reutgard et al., 2014). In laboratory studies, *M. affinis* females have been observed to produce increased numbers of malformed embryos after exposure to sediments saturated with certain chemicals (Eriksson et al., 1996; Jacobson and Sundelin, 2006). In the Gulf of Finland, the frequency of occurrence of the malformed embryos in field populations of the amphipod *G. fasciatus* and other species (*Gammarus tigrinus* Sexton, 1939 and *Pontogammarus robustoides* (Sars, 1894)) was used in a comprehensive environmental assessment of combined exposure to chemical pollution (metals and polycyclic aromatic hydrocarbons) and eutrophication (high levels of dissolved phosphorus and low oxygen levels in water; Berezina et al., 2017).

In our study on the effects of alkylphenol (4t-OP) at a concentration of 0.5 μg/L, we did not find a significant increase in developmental malformations (teratogenic effect); however, significant reproductive disorders were identified (decreased precopulatory activity, that is, the ability to form pairs), signs of the male demasculinization, high embryo mortality rates, and a decrease in the overall fertility of females. Fertility, egg survival, and the number of newborns determine the reproductive success of the population and depend on the size of the female (species) and all environmental conditions that can directly or indirectly affect the period of embryogenesis. Under natural conditions, in the absence of pollution, the level of eggs aborted by amphipods (i.e., the difference between the average number of eggs laid (stages of embryogenesis 1–2) and neonates (newborns)) can be 25–36% (Pöckl, 1993; Berezina, 2011). In this experiment, water pollution with the chemical decreases fertility (the number of embryos per female) almost 2 times in comparison to the control. More than that, over 70% of this number of embryos had serious defects and stopped development; they are not viable. Thus, the survival rate of embryos is very low, less than 10% compared to the control.

The number of amphipod pairs decreased sharply after exposure to 4t-OP at a concentration of 0.5 μg/L. OP is thought to have antiandrogenic activity, which influences aromatase activity and the aryl hydrocarbon receptor function of fish (Bonefeld-Jorgensen et al., 2007). The long-term exposure of males to these concentrations of 4t-OP revealed the demasculinization of males and a decrease in the size of gnatopods, an important organ for controlling females at the precopulation stage, which is important for successfully fertilizing eggs. In particular, the males of the same body size significantly differed between control and experiment variants in the size characteristics of gnatopods I, which are of special importance for the success of mating, and in the increase in the depth of coxal plates IV (female trait). It has previously been shown in natural environments that morphological abnormalities observed in intersex amphipods can lead to reduced mating success (Ford et al., 2003).

The decrease in fertility of *G. fasciatus* is possibly associated with endocrine disorders, although this hypothesis was not tested in this work. A comparison of the fertility of normal and intersex females from a natural population of *Echinogammarus marinus* (Leach, 1816) showed reduced fecundity (i.e., the production of fewer eggs) and fertility in the latter (Ford et al., 2003). Normal females produced approximately 20% more offspring than intersex females. It has been suggested that the causes of decreased fertility are related to the inclusion of testicular tissue in the female ovary and the passive loss of embryos from the brood pouch (marsupium) through deformed coxal plates or the active release of nonviable embryos (Ford et al., 2003).

The reasons for the appearance of individuals with male and female characteristics (intersex) include parasitism (Bulnheim, 1977), bacterial infection (Rigaud and Juchault, 2011), protandrous hermaphroditism (Yaldwyn, 1966), and pollution (Moore and Stevenson, 1991; Ford et al., 2003). According to the size of gnatopods, intersex males formed an intermediate group between normal males with larger gnatopods and normal females with smaller gnatopods (Ford et al., 2004). Therefore, the size of amphipod gnatopods has been proposed as a marker for assessing the number of intersex individuals in a population and the quality of the environment (Ford et al., 2006). In the absence of pollution, the proportion of intersex in many amphipod species is 5–8%, and in the presence of pollution it is higher than 14–15% (Ford et al., 2004). Although a basic level of intersexuality may occur naturally in some invertebrate populations, the reasons for its occurrence are comprehensive and are mainly associated with environmental pollution with estrogen and organic tin compounds that disrupt the endocrine system of organisms, as well as some other reasons (parasitism, genetic abnormalities in sex determination, etc.) (Grilo and Rosa, 2017).

This study confirms the high toxicity of octylphenol even in nonlethal concentrations. Its effect includes irreversible disruptions in the functioning of aquatic organisms, including disturbances in metabolic processes and reproduction. Such reactions, in the long term, can affect the number and even existence of biotic populations and the species diversity of the biota.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All studies were conducted in accordance with the principles of biomedical ethics as outlined in the 1964 Declaration of Helsinki and its later amendments.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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