Standard and experimental drilling fluids' effect on the response of the larvae of bivalve *Hiatella* sp.

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Abstract. Behavior of the larvae of common genus of marine bivalve mollusk Hiatella sp. was tested at the presence of the standard drilling fluid and the water-based drilling fluid containing brown alga Saccharina latissima as biodegradable component. Standard drilling fluid (based on polyanionic cellulose and xanthan gum) is currently used at Prirazlomnaya offshore ice-resistant oil platform. A series of experiments have been performed with live larvae (young and elder stages, ~250 µm and ~350 µm, respectively) at 6.7 and 20 mL/L concentrations of the drilling fluids and in control. Larvae tended to spend more time with closed valves when exposed to standard drilling fluid. Larvae exposed to drilling fluid with *Saccharina latissima* extract were characterized by significantly higher activity comparing both to control group and to standard drilling fluid solution. Elder larvae were more sensitive to standard drilling fluid, being under stress from the very beginning of the experiment (3 hours of exposure) to the end (48 hours of exposure + 24 hours in native sea water). Young larvae tend to be more resistant both to standard drilling fluid and drilling fluid with *Saccharina latissima* extract; this may promote their better survival. Possible biological consequences of using natural and environmentally friendly extract of brown algae on pelagic larvae of benthic species is discussed.

1 Introduction

Despite an increasing share of green and blue energy, global demand for oil and gas is considered to continue, so the mining areas are now spreading and the ocean floor in the shelf areas is actively developed. Ensuring a minimal environmental impact to the surrounding ecosystem is one of the challenges facing offshore oil and gas production. These impacts include sound, increased boat traffic, physical and chemical disturbance of the marine biota [1].

Oil and gas production requires the use of drilling fluid (DF), which properties are largely related to the aquatic or terrestrial environment [2]. Appropriate composition of the DF is very important for successful drilling operations. DF is a mixture of different components of

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various viscosity and properties; all these affect the drilling efficiency [3]. DFs are water-based, oil-based, synthetic, and special DF in regard to the liquid phase. Oil-based DFs were primarily considered the best as it provides high rate of penetration, a reduction in downhole fluid losses, shale stability, and reduced torque and drag [4]. However, the use of oil-based DFs was restricted by environmental protection laws due to both high toxicity and high costs [5; 6]. On one hand, cuttings (small pieces of rock broken away by the drill bit) drilled with non-aqueous DFs (NADFs) are predicted to have minimal impact on local fauna [7]. On the other, DFs contribute to the destruction of rocks, so they have a number of properties that in most cases are harmful to the environment: for example, the sparse benthic filter feeder communities close to the wells have been significantly affected [8]. Indeed, marine biota is one of the most vulnerable elements of the ecosystem, as DFs are partially soluble or form a suspension, degrading habitat for many species [9; 10].

The problem of environmental protection has resulted in an increase in the use of water-based DFs (WBDFs). A lot of commercially available additives belong to non-degradable and environmentally hazardous materials even for WBDFs. Using biodegradable materials as additives in DF may improve significantly this issue. Several eco-friendly local materials (vegetable oils and peels) were already tested successfully as a part of WBDF: almond seed oil [11], crude oil extracted from *Calophyllum inophyllum* L. seeds [12], mandarin peel powder [13], and okra mucilage [14].

The direct observations of benthic communities during deep water drilling are technically difficult and expensive. That is why, some mathematical models have been developed [15]. Although, these models aim on predicting on how do cuttings disperse and so how do total petroleum hydrocarbons re-distribute in the bottom sediments after discharge.

Experimental approach in studying the effect of a factor on a living system at any level in vitro may bring some promising results, which may help in primary assessing the direction of the changes in the studied system in vivo. However, a number of potential issues must be considered when bringing the results to the field. Firstly, exposure to drilling waste lasts for significant time period, thus increasing chronic toxicity probability. Secondly, concentration of contaminant may be considerably lower than lethal concentration, but still causes damage to organisms. Thirdly, acute toxicity tests are usually designed to assess the toxicity of chemical contaminants for regulatory compliance. However, physical disturbances (i.e., smothering or the presence of non-nutritious waste materials in the food supply) and/or organic enrichment impacts may override the 96hr LC50 toxicity effects, so laboratory acute toxicity tests have failed to adequately predict the impact of drilling wastes on benthic communities around drilling platforms: even when acutely toxic diesel-based mud was replaced by mineral oils, this did not reduce the spatial extent of benthic impact zones around drilling platforms in the North Sea, which extended as far as 6 km from a production platform [16].

We hypothesize that experimental drilling fluid has no negative effect on the behavior of Hiatella sp., or this effect is insignificant comparing to standard drilling fluid.

The study aims to assess the effect of two drilling fluids at low concentrations on the behavior of the larvae of common species of bivalve mollusk, *Hiatella* sp.

The goal is to test the standard DF and experimental DF, containing the brown algae *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, 2006 as a biodegradable and therefore environmentally friendly component. The series of experiments were performed to track any alternations in larvae behavior and thus to predict their survival, which finally might affect the overall population success.

2 Materials and methods

The consequences of the possible toxic effects of DFs of various composition were studied in 2021 in a series of experiments with the larvae of the widespread sublittoral bivalve mollusk *Hiatella sp*. The experiments were carried out at the White Sea Biological Station of Zoological Institute, Russian Academy of Sciences (Russia).

Two DFs of different composition were tested:

(1) DF currently applied on the Prirazlomnaya offshore ice-resistant oil platform, prepared on the basis of oil gasol, rosin, bentonite and other chemical components, hereinafter referred to as “ST” (Standard),

(2) an experimental DF developed at the Murmansk Arctic University. The brown algae *Saccharina latissima* is used a biodegradable material, and hereinafter referred to as “BD” (Biodegradable).

The reference group of mollusk larvae was used as a control (hereinafter referred to as “C”). All the procedures performed during the experiment were applied for the control group to avoid additional handling effect on the animals from the groups ST and BD.

Mollusk larvae were sampled with a Juday net, gently removed from a zooplankton sample, and acclimated for 48—72 hours. Then they were placed individually in 2-mL wells of the palette. The DF concentration was 20 mL/L (young stages, ~250 μm) and 6.7 mL/L (young and older stages, ~250 μm and ~350 μm, respectively) (Fig. 1). The larvae behavior was checked in 3, 6, 12, 24, and 48 hours after the experiment started. The animals were then transferred to 2 mL of native sea water for another 24 hours, and their behavior was checked again. Each combination of “larva age–DF–concentration” was tested for 48—56 larvae. In total, 462 larvae were tested individually in three experiments.

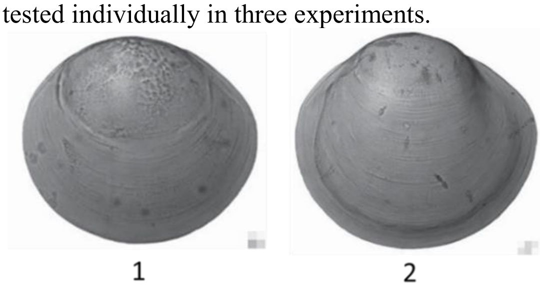

Fig. 1. Larval shell of *Hiaiella arclica* at the early stages of development: 1 — umbo forming stage, young stages ~250 μm; 2 — early pediveliger older stages ~350 μm [17].

The larvae behavior was checked individually for 2—3 minutes for each larva. The behavior modes were first noted as: (0) dead (both stages); (1) swims abnormally (both); (2) stands on cardo (both); (3) lies on the side (both)/ attached (elder stages only); (4) swims normally (both stages)/crawls on the bottom (elder stages only). Then the qualitative characteristics were turned into quantitative, where 4 referred to normal behavior, 0 – to dead larvae (see above). The numerical data were then tested for normality of distribution by Shapiro-Wilk’s test and analyzed using two-way ANOVA.

3 Results

All the larvae were alive except two (one in C and one in ST) after the experiments ended (mortality < 0.5%). The behavior of all larvae exposed to both DFs at both tested concentrations recovered in all animals after their transfer to native sea water. This allowed us further analyzing and concluding on behavioral patterns of larvae in given experimental conditions.

The negative significant effect of the standard DF (ST) was tracked in all experiments (Table 1). Interestingly, it was nearly the same at the lower concentration, 6.7 mL/L and at the threefold higher one, 20 mL/L (Figs. 2—4). Obviously, larvae tend to spend more time with closed valves rather than swimming.

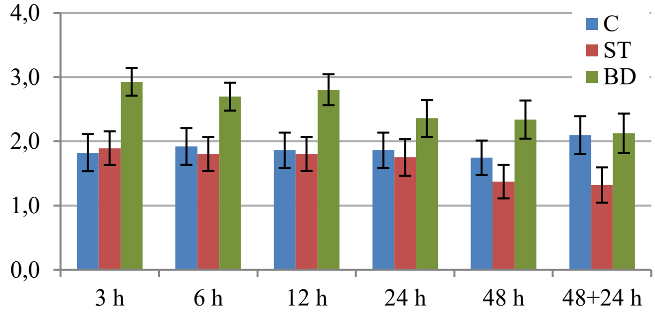

Fig. 2. Effect of DF at 20 mL/L concentration on the behavior of young larvae of *Hiatella* sp. throughout the 72-h experiment.

High concentration of BD drilling fluid affected young larvae to spend more time swimming even comparing to control group (Fig. 2). Effect of BD drilling fluid at low concentration differed for young and elder larval stages. Young larvae tended to spend more time with open valves than elder ones (Figs. 3 and 4). However, when comparing both DFs and control group at 6.7 mL/L concentration for young and elder larvae, similar trend is obvious for all of them, although the differences are significant (Table 1).

It is important to note that the response of the young larvae to ST of 20 mL/L and in C was nearly the same for the first 24 hours of the experiment (Fig. 2). However, larvae exposed to ST were under stress after 48 hours and did not restore their behavior even after transfer to the native seawater (48+24 h).

Table 1. Significance of effect of different drilling fluids (DF) on the behavior of larvae of bivalve mollusk *Hiatella* sp. in experiment (two-way repeated measures ANOVA).

Larval stage and DF concentration	Factor	*SS*	*F*	P_m	F_{lim}	Factor share, %
Young larvae (~250 µm), Standard 20 mL/L	Drilling fluid	**11.43**	**10.92**	**0.00**	**3.86**	**1.9**
	Exposure time	6.58	1.57	0.18	2.39	-
	Factor interaction	10.48	2.50	0.04	2.39	1.7
Young larvae (~250 µm), Algae-based 20 mL/L	Drilling fluid	**45.14**	**42.80**	**0.00**	**3.86**	**7.0**
	Exposure time	8.31	1.97	0.10	2.39	-
	Factor interaction	13.13	3.11	0.02	2.39	2.0
Young larvae (~250 µm), Standard 6.7 mL/L	Drilling fluid	**54.42**	**118.40**	**0.00**	**3.86**	**17.2**
	Exposure time	**11.75**	**6.39**	**0.00**	**2.39**	**3.7**
	Factor interaction	2.79	1.52	0.20	2.39	-

Continuation of Table 1.

Young larvae (~250 µm), Algae-based 6.7 mL/L	Drilling fluid	0.80	1.30	0.25	3.86	-
	Exposure time	**22.37**	**9.07**	**0.00**	**2.39**	**6.28**
	Factor interaction	0.30	0.12	0.98	2.39	-
Elder larvae (~350 µm), Standard 6.7 mL/L	Drilling fluid	**9.52**	**8.28**	**0.00**	**3.86**	**1.6**
	Exposure time	10.89	2.37	0.05	2.39	-
	Factor interaction	5.91	1.28	0.28	2.39	-
Elder larvae (~350 µm), Algae-based 6.7 mL/L	Drilling fluid	**30.75**	**24.13**	**0.00**	**3.86**	**4.6**
	Exposure time	6.35	1.25	0.29	2.39	-
	Factor interaction	9.95	1.95	0.10	2.39	-

Note: SS is the sum of squares of deviations, F – F-ratio, P – level of significance, Flim – critical F-ratio.

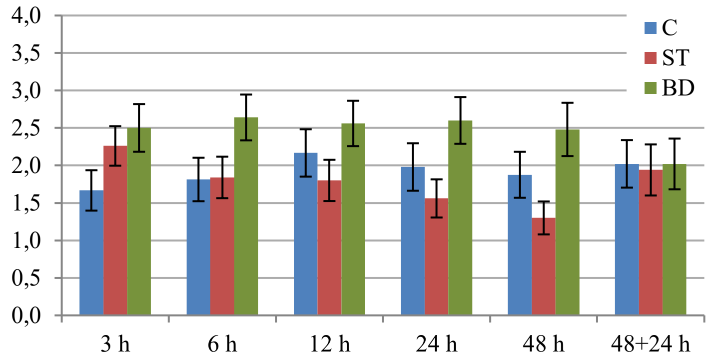

Fig. 3. Effect of DFs at 6.7 mL/L concentration on the behavior of young larvae of Hiatella sp. throughout the 72-h experiment .

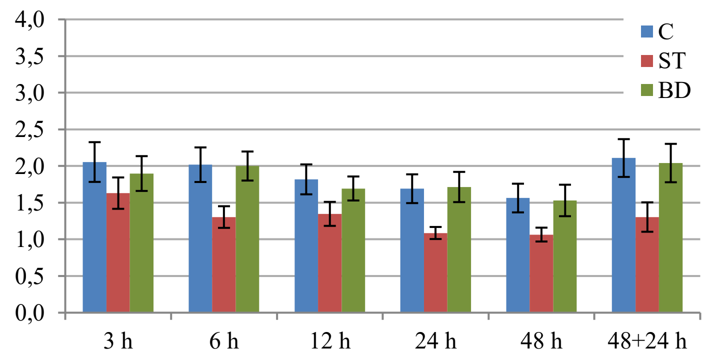

Fig. 4. Effect of DFs at 6.7 mL/L concentration on the behavior of elder larvae of Hiatella sp. throughout the 72-h experiment.

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Interestingly, elder larvae were almost indifferent to BD solution (Fig. 4), whilst ST solution suppressed their activity significantly (Table 1). Most of larvae exposed at ST spent most of time inactive with valves closed.

Elder larvae were also more sensitive to ST at 6.7 mL/L concentration comparing to young ones (Figs. 3 and 4). While young larvae have restored their behavior to normal (similar to C) after 24 hours in native seawater (Fig. 3), elder ones were under severe stress from the very beginning of the experiment until the very end of it (Fig. 4).

4 Discussion

A number of studies dated almost four dozen years ago referred mostly to experiments, where acute toxicity of a used seawater with DF or even a DF component was tested [18]. In these studies, acute toxicity of the DF aqueous fractions was primarily due to volatile soluble organic, whilst that of the suspended solids phase, and of layered solids phase solutions, to the smothering action of fine particles in DF for marine polychaetes (*Neanthes arenaceodentata*, *Ophryotrocha labronica*, *Dinophilus* sp., and *Ctenodrilus serratus*), crustaceans (*Penaeus duorarum*, *P. aztecus*, *Mysidopsis almyra*, and *Palaemonetes pugio*), and mollusks (*Donax variabilis texasiana* and *Aequipecten amplicostatus*). It was concluded that discharge of a used DF with chrome lignosulphonate from offshore platforms would not likely cause significant damage to benthic, demersal, or pelagic marine animals [18]. Later studies on long-term toxic effect of DF on adult sea scallops, Placopecten magellanicus, revealed that threshold waste concentrations causing reductions in somatic and/or reproductive tissue growth in the laboratory under environmentally representative conditions [16]. The authors concluded that chronic intermittent exposure of these mollusks even to low concentrations of operational DF wastes, characterized by acute lethal tests as practically non-toxic, could affect growth, reproductive success, and survival significantly.

Although WBDFs are considered to be the least harmful, their composition still remains under discussion. On one hand, sublethal screening tests of water-soluble fraction of drill cutting on adult blue mussel, *Mytilus edulis*, bring promising results, when their biological effects on fauna might be assumed as a toxic stress of low intensity [19]. In addition, when testing WBDF effect on the survival rate and linear growth rate of the same mollusk species, standard WBDF (based on barite and carboxymethyl cellulose) led to the 100% death after 9 days of exposure even at minimal concentration, while the experimental WBDF, similar to that used in the present study, caused no death [20; 21]. DF not containing barite and carboxymethyl cellulose may be considered safe for marine bivalves; their components seemed to be easily degradable, since some of them may be used as a food source by some detritus-feeding invertebrates [21].

Such environmentally-oriented approach has been followed until recently. Indeed, high concentration of DF is rarely found at a distance from the drilling well, but DF plume of low concentration may affect all the parameters described above but also the behavior and thus survival of the most vulnerable, larval stages. In our study we attempted to assess the direct and short-term effect on low concentrations of standard and WBDF enriched with biodegradable component (brown alga *Saccharina latissima*). Obviously, there is a pronounced effect of both DFs on the bivalve larvae behavior observed in our study; however, the patterns differ. At the presence of BD drilling fluid larvae are more active. On the one hand, this may have positive effect, since young larvae swims actively so they have more chance to escape the DF plume zone. On the other hand, they may be affected much more by toxic compounds when their valves are open during swimming and aerobic breathing. Such an effect of BD drilling fluid may be partly explained by the sensitivity of Hiatella larvae to repellent compounds of *Saccharina latissima*, preventing the settling and thus fouling of algae thallus by relatively large mollusks [22]. In addition, BD drilling fluid

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may cause negative consequences for larvae as well pushing them to keep their valves open for longer time and thus increasing their exposure to other, probably more toxic, compounds of DF. However, serving a natural repellent, *Saccharina latissima* extract may play a positive role in preventing young (=vulnerable) larvae in staying too long in the DF plume zone, promoting them seeking for a safe (repellent-free) environment. Definitely, this issue demands further studies involving physiological and biochemical analyses.

5 Conclusion

Unexpected stimulating effect of water-based drilling fluid containing brown algae (*Saccharina latissima*) extract on the behavior of marine bivalve mollusk larvae (Hiatella sp.) has been found. Larvae exhibited increased activity comparing both to standard drilling fluid solution and to the control group. Elder larvae are more sensitive to standard drilling solution, being under severe stress from the very beginning of the experiment. We suggest anti-fouling properties of this natural compound of local origin may play positive role in preventing the larvae for long-term exposure to other toxic components of a drilling fluid, promoting their active search for safe environment. The study emphasizes the need to choose the drilling fluid(s) with minimal negative effects both on benthic and pelagic fauna for future oil and drilling near sensitive environments (for example, near protected areas).

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