FIRST CASE OF IMPOSEX IN *NEPTUNEA CONVEXA* (GASTROPODA: BUCCINIDAE) FROM BATHYAL DEPTHS (SEA OF OKHOTSK, >1400 M)

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Owing to its worldwide use as an anti-fouling agent, tributyltin (TBT) is a common contaminant of marine ecosystems. Its wide distribution, high hydrophobicity and persistence have raised concern about bioaccumulation, potential biomagnifications in food webs, and adverse effects on the environment and human health. The most frequent and acute effect of TBT is found in gastropods, usually living in shallow waters, rarely at depths more than 100 m. This study reports about the first case of imposex in a deep water buccinid whelk *Neptunea convexa* collected at 1437 m in the Sea of Okhotsk. Among five collected specimens, the two were imposex females at the 1st stage of imposex development, while the rest three were males with normally developed penises. Most probably, TBT entered the whelk's body by eaten benthic organisms, which feed on detritus with traces of TBT, but other reasons, such as heavy metal pollution, are also discussed.

Key words: imposex, pseudohermaphroditism, Neptunea, Gastropoda, TBT, tributyltin, heavy metals, pollution

I. INTRODUCTION

Imposex or pseudohermaphroditism (appearing of male reproductive system characters in females of dioecious gastropods), was described in 70th years of the 20th century. At the moment, the most frequent and acute effect of TBT is found in gastropods: more than 150 mollusk species, predominantly belonging to Neogastropoda, have been reported to be affected [1]. It was shown that imposex has been caused by chemicals based on tributyltin (TBT), a common contaminant of marine ecosystems used as an anti-fouling agent. Its wide distribution, high hydrophobicity and persistence as well as capacity for bioaccumulation and potential biomagnification in food webs, produce adverse effects on the environment and human health. TBT causes endocrine disruption with androgen levels higher than normal which lead to masculinization in females. During last decades, imposex have been using as environmental pollution indicator. The majority of imposex cases were observed in sea port areas, in shallow waters not deeper than 100 m.

Our study reports about the first case of imposex in a deep water buccinid whelk *Neptunea convexa*.

II. MATERIAL AND METHODS

The material for the study was obtained in 61 cruise of R/V "Academik M.A. Lavrentiev" (2013). The main objective of the cruise was investigation of the unique deep-water benthic

ecosystems, formed in active fields on sites of cold seeps, with large amount of gas in their composition, usually methane. In particular, the zones of gas emanation with high methane concentration were examined in Deryugin Basin (the Sea of Okhotsk) at depths up to 1500 m (Fig. 1A). In point with coordinates 54°00.6′ N, 146°25.6′ E (depth 1431–1448 m, 2.3°C, salinity 34.4‰), by means of remotely operated underwater vehicle, 5 big specimens of *Neptunea convexa* (shell on Fig. 1B), and one specimen of *Buccinum pemphigus* were sampled. For comparison, we have studied buccinids, collected near North Kurile Islands (Paramushir Island, 50°30.9′ N 155°18.4′ E, 778 m) (Fig. 1A), with similar zones of gas emanation, i.e. *N.insularus* (1 specimen), *Buccinum pemphigus* (1 specimen), *Ancistrolepis grammatus* (1 specimen).

Elemental analysis of samples was carried out in the Analytical certification test center of the Institute of Microelectronics Technology and High Purity Materials, Russian Academy of Sciences (Chernogolovka, Russia). Concentrations of Fe, Ni, Cu, Zn, Ag, Cd, Hg, Pb and U in the samples were determined by atomic emission (iCAP-6500, *Thermo Scientific*, USA) and mass spectral (X-7, *Thermo Elemental*, USA) analysis techniques.

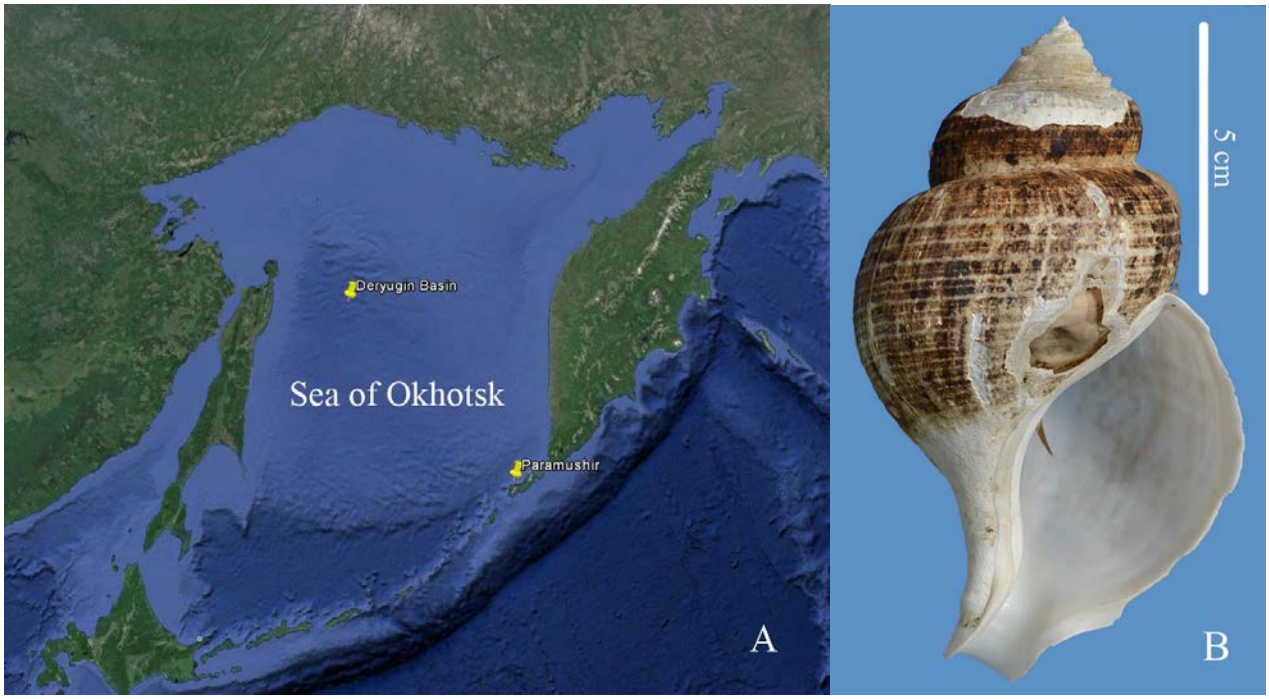

Fig. 1. A – map of the Sea of Okhotsk with sampling sites; B – shell of *Neptunea convexa*.

III. Results

Morphology

Among collected *Neptunea*-whelks, there were 3 males with normally developed penises (Fig. 2) and 2 females with normally developed gonads and capsule glands (Fig. 4C) and at the same time, with small penises on the heads behind the right tentacle (Figs. 3, 4 A-B). Presence of penises is a characteristic feature of imposex. Very small size of penises (< 2 mm) and absence of any characters of seminal duct allow indicate the stage 1 of imposex development [2]. One specimen of *Buccinum pemphigus*, collected in the same locality appeared a normal female without a penis.

All buccinid whelks from Paramushir Island appeared normal females without penises.

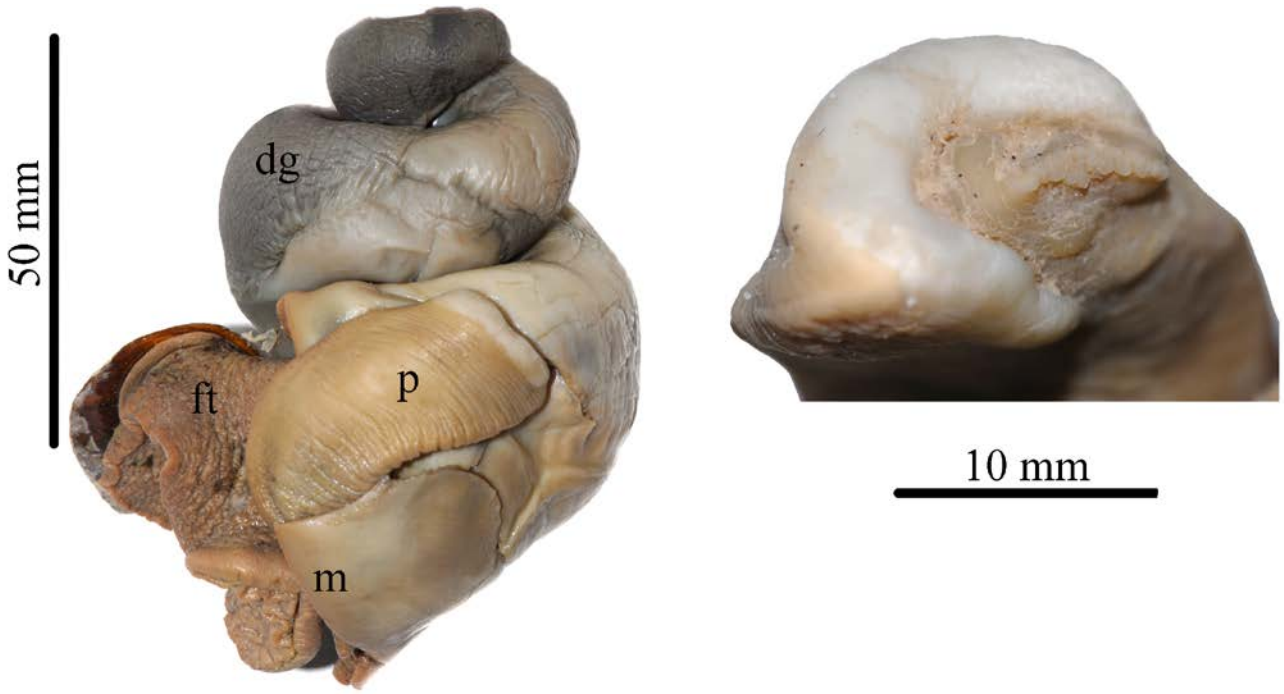

Fig. 2. Reproductive morphology of normal male of Neptunea convexa*. Left – whole soft body, extracted from the shell; dg – digestive gland, ft – foot, m – mantle, p – penis. Right – apical part of the penis.*

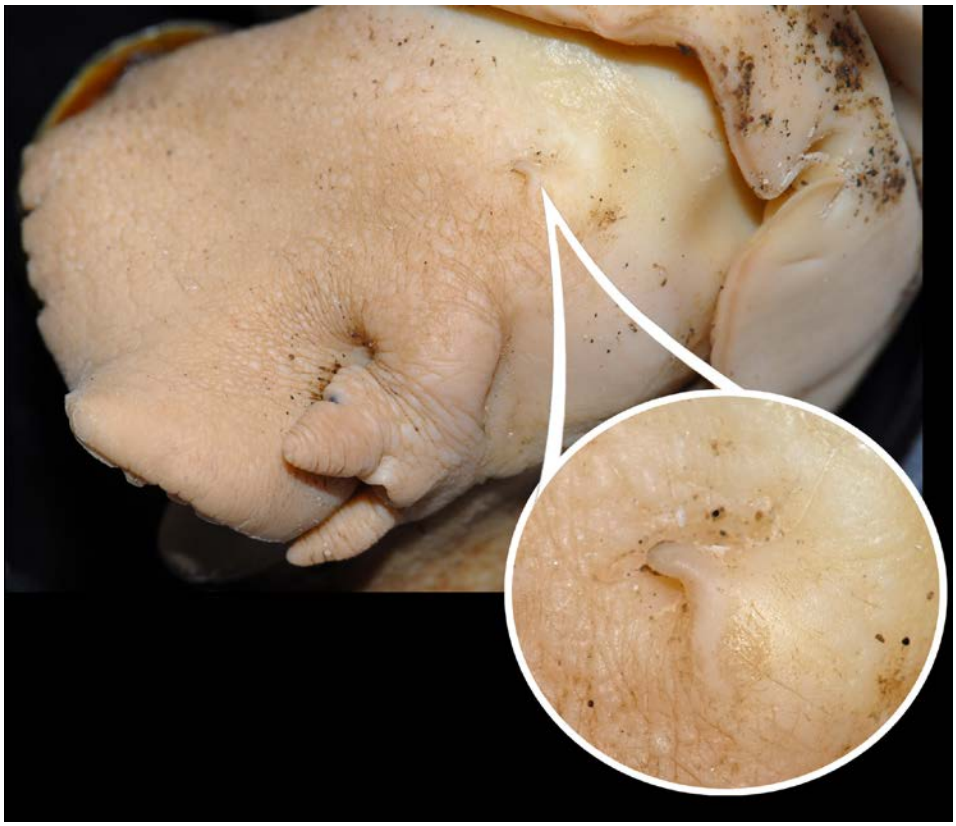

Fig. 3. Imposex female 1 of N. convexa*. Penis enlarged in the circle*

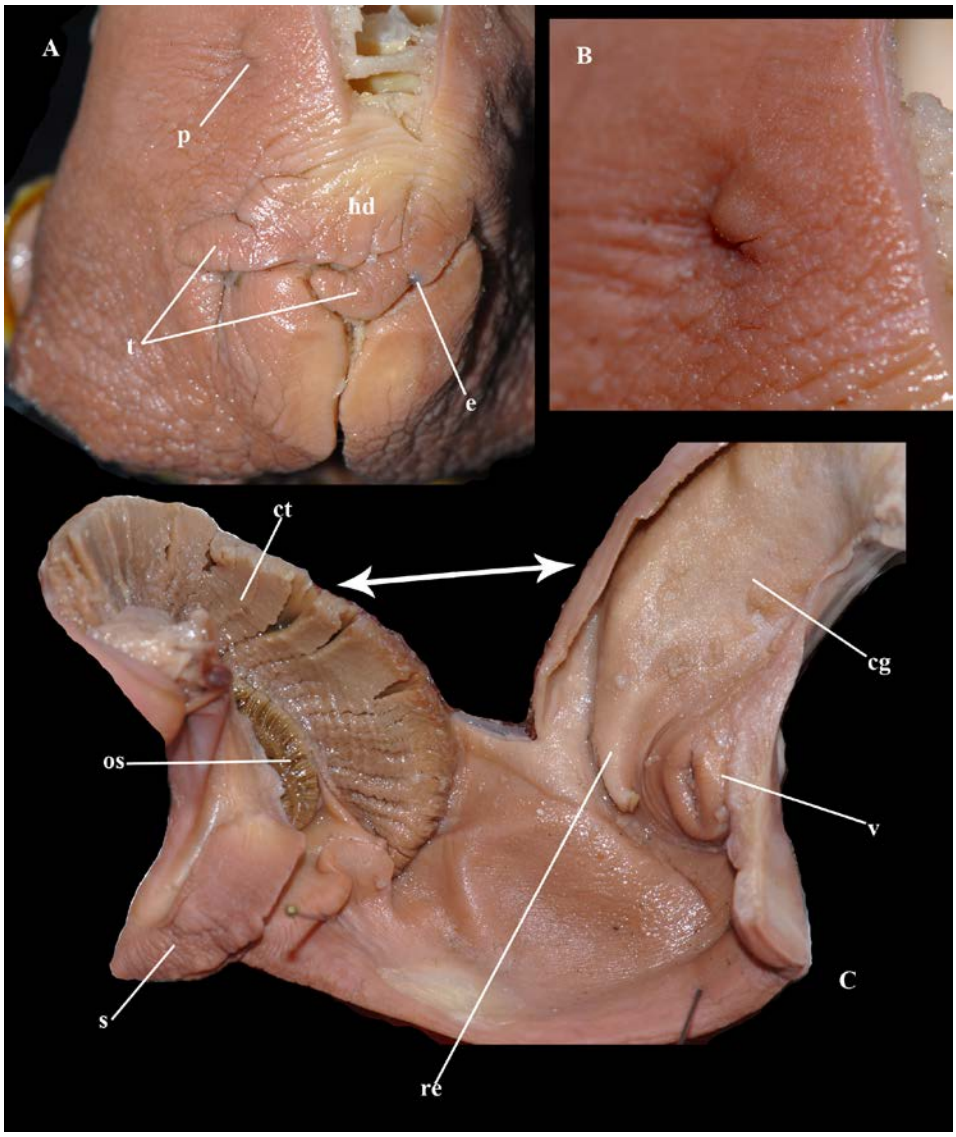

Fig. 4. Imposex female 2 of N. convexa. *A – cephalopodium, e – eye, hd – head, p – penis, t – tentacle. B – penis enlarged. C – mantle, cg – capsule gland, ct – ctenidium, os – osphradium, re – rectum, s – siphon, v – vagina.*

Heavy metal analysis

Heavy metal analysis of the whelks revealed no signs of tin in their foot and digestive gland, but there were unusually high, even for hydrothermal vents, concentrations of zinc and cadmium (10573 and 1407.3 mcg of zinc in digestive gland and foot respectively, 373.3 and 43.8 mcg of cadmium in digestive gland and foot respectively) (Table 1). Since the material was fixed not properly for metal analysis (in 70% alcohol instead of freezing), we also analyzed alcohol in which the material was stored to check if the metals could diffuse to molluscan tissues from contaminated alcohol. As a result, we found 10-20 times lower meanings of zinc and cadmium in alcohol.

The presence of other heavy metals was registered, but their concentration did not exceed average meanings for other investigated mollusks. Concentration of Pb varied from 0.25 to 2.00 µg/g, concentration of Hg varied from 0.026 to 0.43 µg/g, concentration of U varied from 0.18 to 14.5 µg/g.

Table 1. Concentration of heavy metals in foot and digestive gland of buccinids from the Sea of Okhotsk, 770-1438 m.

Species	Sampling site	Foot, µg/g						Digestive gland, µg/g					
		Zn	Cd	Cu	Ni	Fe	Ag	Zn	Cd	Cu	Ni	Fe	Ag
Neptunea convexa, imposex female1	Deryugin Basin	1763	53.6	100	2.1	210	3.4	4594	314	164	1.8	691	22.4
Neptunea convexa, imposex female2	Deryugin Basin	618	24.2	58.7	5.8	72.2	2.3	11292	242	117	8.5	1170	9.3
Neptunea convexa, normal male1	Deryugin Basin	1568	47.4	103	2.5	203	2.8	11558	564	155	1.3	828	7.7
Neptunea convexa, normal male2	Deryugin Basin	1680	49.9	107	3.0	296	5.4	14848	373	340	2.4	2091	19.1
Alcohol	Deryugin Basin	448	3.3	366	0	0	0	919	8.4	415	0	0	0
Buccinum pemphigus	Deryugin Basin	591	24.8	97.4	11.7	66	2.3	6833	145	403	15.3	935	6.2
Buccinum pemphigus	Paramushir	141	33.9	62.4	1.3	143	1.5	-	-	-	-	-	-
Neptunea insularis	Paramushir	156	15.1	122	0.77	247	0.78	4392	267	253	1.7	1541	8.4
Ancistrole-pis grammatus	Paramushir	198	10.8	42.0	1.9	903	0.36	-	-	-	-	-	-
Alcohol	Paramushir	-	-	-	-	-	-	202	0	0	0	34.1	11.6

IV. Discussion

Neptune whelks are typically dioecious, not having sex change and planktonic larvae during lifetime. Their feeding is still poorly studied, but there are evidences of predation on benthic invertebrates as well as scavenging [2, 3]. More recent determinations of the half-lives of TBT have shown them to be as high as 8 years in sediments and 4–17 years in the bivalves; the last are usually filtrators and are capable of bioaccumulation. These facts give reason to believe that TBT (which traces (Sn) we failed to detect via metal analysis probably due to inappropriate fixation of the material) entered the whelk's body by eaten benthic organisms, which feed on detritus with traces of TBT. At the same time we cannot exclude other reasons of imposex, such as high concentration of other heavy metals (especially zinc and cadmium). Concentration of heavy metals in samples from Deryugin Basin was in majority of cases lower than in those from Paramushir. And average concentration of heavy metals, especially zinc and cadmium, was significantly higher than in other mollusks with high metal pollution, for example, those from Peter the Great Bay (the Sea of Japan) [4] and even hydrothermal vents [5]. There are evidences that lead pollution is connected with pseudohermaphroditism in *Pugilina* (Buccinoidea) [6], and copper induced imposex in experiments [7]. The imposex may be also a consequence of parasitic infection. Thus, larval stages of a

trematode *Stephanostomum baccatum* were found in digestive gland of *Buccinum undatum*, and cadmium pollution was shown to increase susceptibility of snails *Biomphalaria* to infection with trematodes *Schistosoma* [8, 9].

IV. Acknowledgment

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V. References

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