MORPHOLOGICAL CHANGES IN MARINE PLANCTONIC ANIMALS *CHAETOGNATHA* UNDER RADIATION EXPOSURE

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Abstract

The subject of investigation were morphological changes in the marine planctonic animals, Chaetognatha (arrow worms), exposed to anthropogenic radiation. The animals were taken in the epicentre of the radioactive spot of the Chazhma Bay (the Sea of Japan). Morphological deviations were determined under a hand magnifying lens and a microscope, while tissue changes were studied on histological sections. The studies have revealed that radiation causes damage to the skin epithelium, changes in fin plates, separation of the skin epithelium from muscles, separation of teeth apparatus from the body surface, damage to the intestine. These changes are characteristic solely of radiation pollution and are not encountered under any other damaging effects such as those of geophysical factors, heavy metals pollution, influence of ultrasound. Anomalous animals preserve their viability, and this makes it possible to use the chaetognaths for the bioindication of radiation pollution of marine waters.

Key words: *Chaetognatha, morphology, histology, ecology, radiation*

1. INTRODUCTION

Antropogenic impact on marine ecosystems has been notably increasing globally, which has been reflected as a concern in a number of international documents of UNESCO, IUBS, and DIVERSITAS. These processes have already resulted in changes of marine ecosystems, and reduction of biodiversity and reserves of bioresources worldwide (Adrianov 2004). Marine pollution by radionuclides constitutes a serious health hazard to humans due to consumption of contaminated organisms with food, and may cause a number of serious diseases, including cancer. The sources of radionuclides in the marine environment are radioactive waste disposal in the World Ocean, which used to be a common practice of all leading nuclear powers during many years, sunken nuclear submarines, discharge of radioactive liquids from ships and submarines, etc (Sivintsev et al. 2005). Studying the biota of ecosystems exposed to pollution is crucial if we are to solve problems of ecological safety and to monitor environmental conditions (Adrianov 2004).

The object of this study was to evaluate morphologic changes in planktonic organisms inhabiting Chazhma Bay (Peter the Great Bay, the Sea of Japan), which was exposed to routine pollution typical for urban areas and was affected by significant radioactive contamination as a result of an accident. Chazhma Bay is located in the northeast part of Peter the Great Bay. A distinctive ecological feature of Chazhma Bay was a radionuclide contamination of its bottom sediments resulting from a submarine's nuclear reactor accident in 1985 (Sivintsev, Vysotsky & Danilyan 1994). The major component of the radioactive pollution was corrosive Co-60 (>99%), and partially - a product of nuclear decay of Cs-137. In the accident zone, the maximal specific activities of Co-60 and Cs-137 in the bottom sediment layers were quite high, as they exceeded 107 and 103 Bq/kg, respectively. Therefore, the benthonic sheet of water was exposed to intensive radiation (Belan 1998; Sergeev et al. 2001). Radiometric studies demonstrated that the intensity of gamma radiation in water in some places of the accident zone decrease to the background value only at 2 m above the bottom. Thus marine organisms of the benthonic layer and of the bottom sediments in the zone of the accident have been exposed to an excessive radiation pressure.

Analysis of plankton from Chazhma Bay revealed striking morphological changes in planktonic animals, the chaetognaths. The chaetognaths, also known as arrow worms, (phylum Chaetognatha) are mass and typical representatives of plankton (Kasatkina 1982, Pierrot-Bults & Nair 1991). These free living marine animals inhabit water with a salinity not lower than 8 ‰. The chaetognaths are planktobenthic, that is they are able to freely move in the water, but during certain periods of life cycle stay close to the bottom (Kasatkina 1982). Ethological observations show that chaetognaths laying eggs (animals of 4th and 5th stages of maturity) and thus staying at the bottom are the most exposed to the radiation, whereas immature chaetognaths freely moving in the water at varying distances from the bottom are least affected.

The chaetognaths are elongated animals of a sagittal form (Slabber 1769). The size of mature individuals ranges from 5 to 120 mm (Kapp 1991). The body of the chaetognaths is subdivided into three compartments: head, trunk, and tail. The upper part of the head contains the head ganglion (brain), grasping spines and teeth. On the dorsal side of the head, behind the brain, there is a pair of eyes, and, on the ventral part – the mouth. Paired fins are located on the sides of the body, and at the terminal end there is an unpaired tail fin. Each fin consists of a transparent plate, usually with rays. The surface of the body is covered by a stratified epithelium with a unique ultrastructure, where the cells are interconnected by special bridge-like junctions (Stolyarova & Kasatkina 1988, 2001, Kasatkina & Stolyarova 2010). Most of the chaetognaths species (*Parasagitta elegans, P. arctica, P. liturata, Sagitta kussakini, Leptosagitta collariata, Aidanosagitta crassa, Pterosagitta draco, Serratosagitta pacifica*, etc.) have skin epithelium thickenings called the alveolar tissue which can cover particular parts of the body. Muscle tissue is developed in all three compartments of the body, and in trunk and tail it forms four longitudinal cords adjacent to the skin epithelium (Kasatkina 1982). Representatives of Chaetognatha, both colored and colorless, are transparent (Bieri 1966, Tokioka & Bieri 1966).

The aim of this study was to analyze morphological changes in Chaetognatha from the radioactive spot in the area of Chazhma Bay accident. To test for the specificity of findings observed, we have exposed living chaetognaths to the radioactive bottom soil from the bay. We have also addressed the question whether naïve and radiation-exposed chaetognaths have similar behavior and viability.

2. MATERIALS AND METHODS

Mature chaetognaths of several species used in this study were collected from the epicenter of the radioactive submarine accident area in Chazhma Bay. Samples of plankton were taken by research associates of Laboratory for Radioecology, Pacific Oceanological Institute (Vladivostok, Russia) during different seasons in 1999-2005. The samples were taken in a vertical fashion throughout the water depth from the bottom (8-18 m) to the surface. Juday net with the entrance opening of 37 mm in diameter was used. Chaetognaths were picked from a mixed plankton sample with angled forceps from below, or with a pipette. This allowed avoiding mechanical damage to the animals during their isolation (Kasatkina & Sergeev 2004). The animals were fixed in 4% formalin, analyzed with a magnifying glass and under a microscope, and processed for histological sectioning. For that, the material was embedded in paraffin. The 5-7 micrometer thick sections were stained with Boehmer or Mayer hematoxylin and eosin, also Nissle method was used. Histological preparations were examined using a light microscope Leica DME (Leica, Germany), and images were taken with a digital camera Leica EC3 (Leica, Germany).

In a separate experiment, living chaetognaths were exposed to the radioactive soil transported from Chazhma Bay. For that, aliquots of plankton containing different species of Chaetognatha (such as *Parasagitta elegans*, *P. liturata*, *Sagitta kussakini, Leptosagitta collariata* and other natural inhabitants of the area) were collected from 20 catches in ecologically clean water of Amur bay according to the method of Kasatkina et al. (Kasatkina, Shumilin & Goryachev 1991). From the aliquot, the chaetognaths were manually picked using a pipette and a Koch dish and divided into two groups (experimental and control), 50 animals in each. Experimental animals from an ecologically safe

region together with ecologically clean sea water were placed in an aquarium with radioactive soil, whereas the controls were held in ecologically clean water and soil.

The experiment spanned three weeks. Throughout this period of time the behavior and morphology of both experimental and control animals were daily visually observed. Seven days into the experiment, some animals (2-3 from each group) were fixed in 4% formalin for an examination. Each fixed animal was assessed with a field magnifying glass at magnification x4-6 (an average size of the animals was 15-20 mm), and with a microscope to reveal morphological changes in the chaetognaths exposed to radiation. After the lapse of three weeks, all the remaining animals were removed from the aquarium and fixed in 4% formalin. Viability of both groups was also compared.

3. RESULTS

3.1. Major morphological deviations at the anatomical level

The chaetognaths exposed to radiation were assessed for the integrity of the body as a whole, body turgor, state of the skin epithelium, state of fins and digestive tube, eye symmetry, and preservation of muscle tissue.

Damage to the fin plate. Destruction of the fin plate is the most common anomaly in the chaetognaths. While normally a fin plate has an even edge (Fig. 1a), in affected animals the edge between the fin rays is damaged. The fin rays remain intact and most often protrude beyond the fin plate (Fig. 1b). Sometimes, the plate destruction results in fin edges resembling a rooster's comb rather than a fringe (Fig. 1c). This is particularly characteristic of abnormal chaetognaths from Chazhma Bay. It should be mentioned that mechanical damage to the animals never produces the rooster's comb feature, since mechanical force damages not just the rays-free zone (and sometimes, the whole fin plate), but the rays themselves.

It is quite striking that in all species that have regular dense rays, the latter remain intact in all fins of affected individuals. This may be due to the fact that the rays have a more resistant biochemical content, than the fin plate (Duver & Salat 1991). So only the fin plate is destroyed, which accounts for the damage patterns described above. These abnormalities are observed in species having dense numerous rays in the fin plates, such as *Leptosagitta ocis* Kassatkina et Sergeev 2004, *L. exigua* Kassatkina et Sergeev 2004, *L. icis* Kassatkina et Sergeev 2004, *L. nudata* Kassatkina 1973, *L. collariata* Kassatkina 1973, *L. uschakovi* Kassatkina 1973, *L. eris* Kassatkina et Selivanova 2003, *Aidanosagitta scarlatoi* Kassatkina 1971, *A. macilenta* Kassatkina 1971, *A. acus* Kassatkina et Selivanova 2003, *A. pilum* Kassatkina et Selivanova 2003, *A. murex* Kassatkina et Selivanova 2003, *A. bella* Kassatkina et Selivanova 2003, *A. venusta* Kassatkina et Selivanova 2003, and *Parasagitta septicoela* Kassatkina 1971. In some species (genus *Oligoradiata*), the absence of regular rays is a taxonomic feature and, hence, is not an evidence of damage. Destruction of the fin plate edge in the species that do not have regular straight fin rays results in the fin acquiring a shape of a narrow strip, or even in its total loss together with its irregularly shaped, winding rays.

Damage to the alveolar tissue. The surface of the alveolar tissue layer becomes uneven (Fig 1d), which can be easily seen under a magnifying lens. This may probably result from a partial loss of the alveolar tissue cells. This kind of abnormality is seen in the species with well-developed alveolar tissue, such as *Leptosagitta ocis*, *L. exigua*, *L. icis*, *L. nudata*, *L. collariata*, *L. uschakovi*, *L. eris*, *Aidanosagitta scarlatoi*, *A. macilenta*, *A. acus*, *A. pilum*, *A. murex*, *A. bella*, *A. venusta*.

Damage to the intestine. Visually, changes in the intestine look like the local destructions of the wall (Fig. 1e), up to complete disappearance of a gut portion in some cases. Mechanical injury to the gut during the collection of the animals never occurs. In theory, such injury might be possible if, during the sorting procedure, the animal suffered from an excessive pressure of the forceps. However, in this case, external tissue damage would be also observed. Among the chaetognaths gathered from Chazhma Bay, there were those that had a damaged gut while preserving absolutely normal outer tissues (*P. septicoela*, *L. exigua* and others).

Disconnection between teeth apparatus and the body. In some animals, the grasping spines and teeth partially detach from the head. In extreme cases, the massive apparatus of grasping spines and teeth is completely separated from the head which makes representatives of Chaetognatha look very unusually, as if they have "a bald head" (Fig. 1e) (Kassatkina & Sergeev 2004). This anomaly was observed among the representatives of *Leptosagitta* (*L. exigua*, *L. icis*) and *Parasagitta* (*P. septicoela*).

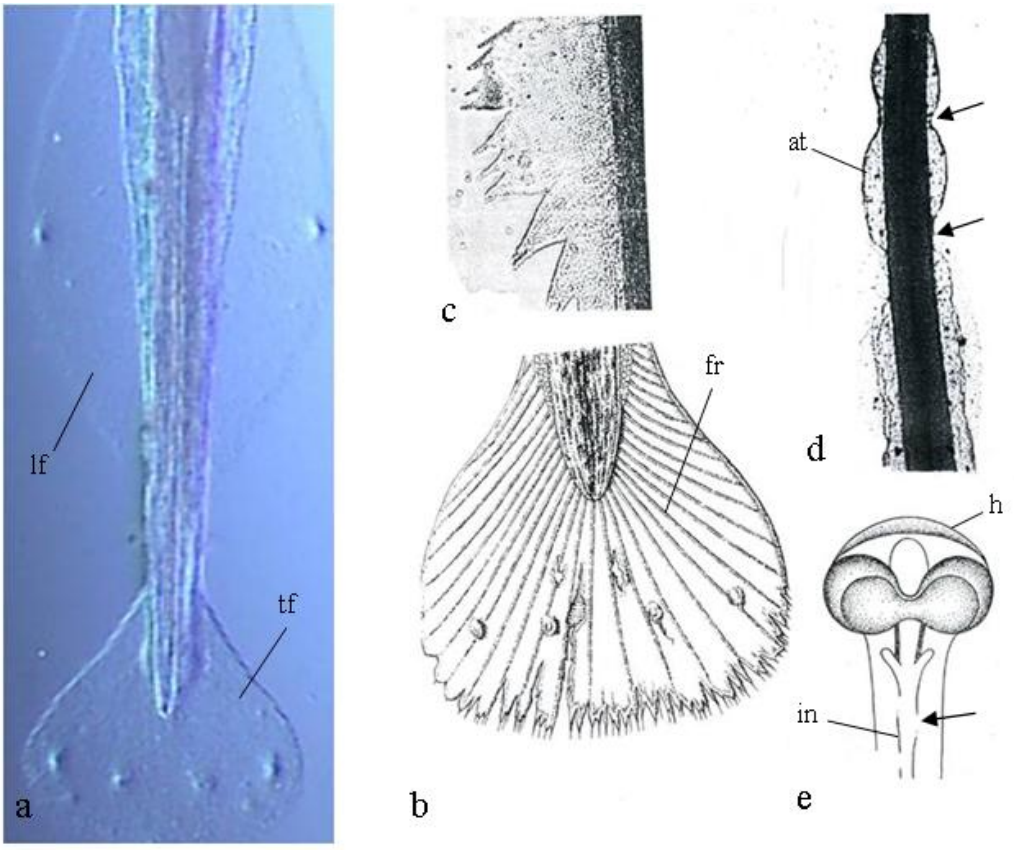

Figure 1. Body parts of chaetognatha under normal conditions (a) and after radiation exposure (b - e).

a - posterior portion of the body (photo of living animal); b - tail fin, c - lateral fin, d - portion of trunk, e - anterior portion of the body (schemes).
Indications: at - alveolar tissue, fr - fin rays, h - head without teeth apparatus, in - intestine, lf - lateral fin, tf - tail fin. Arrows indicate local destructions in alveolar tissue and intestine.

3.2. Morphological deviations observed on histological sections

It is important to stress, that the changes described below cannot be visualized without histological methods. The most striking morphological deviations observed at the histological level (as distinct from the anatomical one) are as follows.

Changes in skin epithelium. Stratified skin epithelium covers the animal body, normally forming an uninterrupted layer (Fig. 2a,b). Abnormal individuals are characterized by notable changes in the areas where the epithelium forms the "alveolar tissue" – one of the most remarkable anomalous features concerned. Numerous portions of this tissue demonstrate detachment of cells from the common layer, probably, due to the destruction of cell junctions (Fig. 3 a,b). As a result, the smooth surface of the epithelium becomes rough. In the cells, the nuclear chromatin is significantly condensed. The basement membrane of the skin epithelium looks folded and there appear areas of muscle detachment.

Changes in the intestine. The intestine normally is composed of large and often vacuolized epithelial cells (Fig. 2a,b; Fig. 4a). In affected animals it not only loses its axial location, but also undergoes reduction in size and deformation (Fig. 4b). A cross section reveals intestine as a cell cluster of an irregular shape or in the shape of a flattened bubble (Fig. 4b). Cells of the intestinal epithelium grow significantly smaller, and their borders and intestinal lumen become hard to detect or impossible to see. Cells of the intestinal epithelium, which are normally large and have a transparent cytoplasm, look compressed and dense.

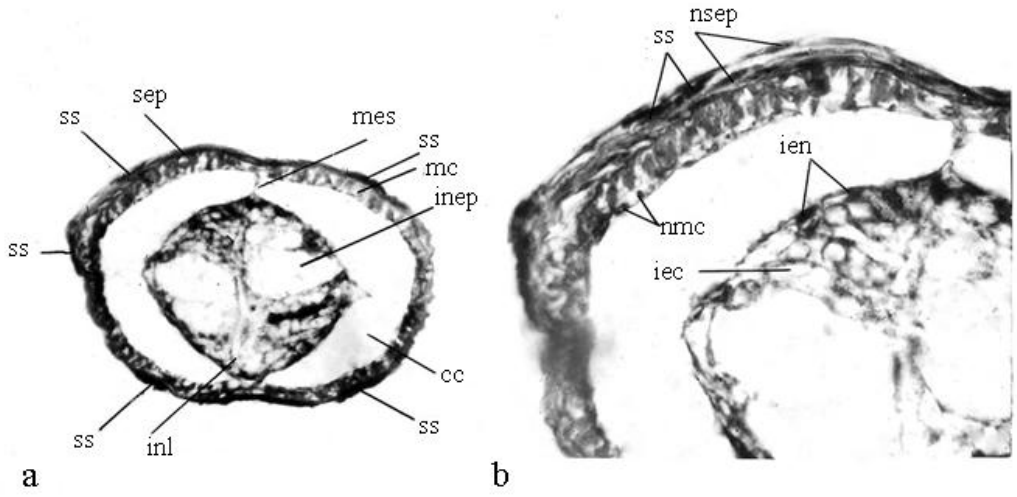

Figure 2. Histological section of normal representative of Chaetognatha *Aidanosagitta macilenta*.

a - transverse section at the level of middle gut, b - portion of body wall and intestine. Indications: cc - coelomic cavity, iec - cytoplasm of cells of intestinal epithelium, ien - nuclei of cells of intestinal epithelium, inep - intestinal epithelium, inl - intestinal lumen, mc - muscle cord, mes - mesenterium, nmc - nuclei of cells of muscle cord, nsep - nuclei of cells of skin epithelium, sep - skin epithelium, ss - sensory sites.

Staining: Mayer hematoxylin. a - ob. 40x, ok. 10x; b - ob. 100x, ok. 10x

Changes in the axial body symmetry. Transverse section of normal animals reveals an almost oval body shape (Fig. 2a, 4a). This is provided by a geometrically regular position of muscle cords of the body wall – both dorsal and ventral, between which in a central position is the gut. Abnormal animals lose this symmetry to a greater or lesser degree (Fig. 4b). This goes along with the loss of the typical shape of every muscle cord, with their deformation and a shift towards each other, and the loss of borders between them. Deformation of the body wall leads to the dislocation of the gut, which may be shifted from the central position, while dorsal and ventral mesenteries connected with the gut also shift and are hardly visible.

Detachment of the ventral ganglion. The ventral nerve ganglion, located in the depth of the epithelial layer (Fig. 4a), progressively loses its contact with the muscles (Fig. 4b).

3.3. Direct radiation exposure experiment

Healthy animals responded to radioactive soil immediately: the thickness of alveolar tissue reduced sharply, and the animals staying in upper water layers went down to the bottom. In spite of the obvious damage to the alveolar tissue, the animals did not die, continued to move, and maintained their viability throughout the same period of time, as the animals in the control tank. Seven days later, all experimental animals, both living and fixed, revealed the damage to the alveolar tissue and to the narrow anterior strip of fins, while tissues of control chaetognaths remained intact. Two weeks after

the beginning of exposure, three animals with lost chitin apparatus (grasping spines and teeth) could be seen. No chaetognaths in the control group demonstrated damages, which we observed in those from Chazhma Bay.

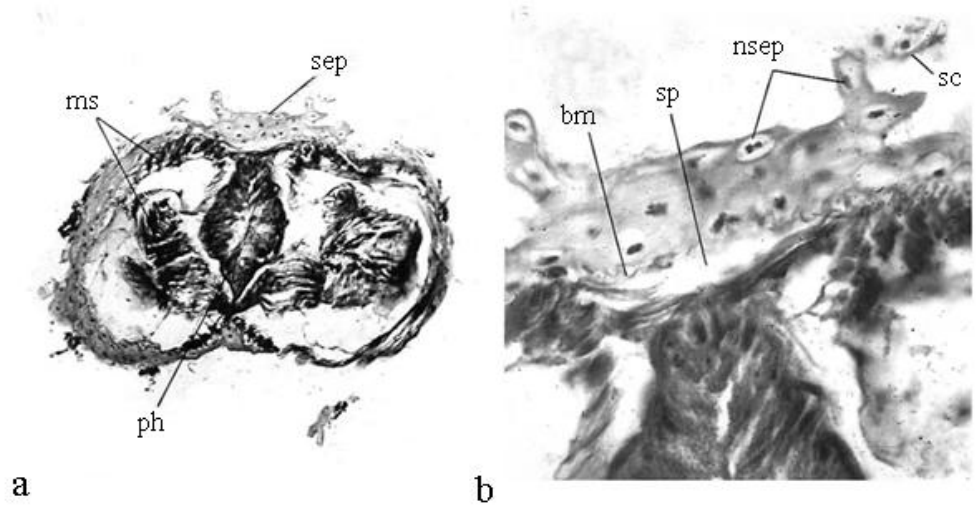

Figure 3. Histological section of *Aidanosagitta* from radioactive contaminated region

a - transverse section at the level of anterior gut, b - portion of body wall.
Indications: bm - basement membrane, ms - muscles, nsep - nuclei of cells of skin epithelium, ph - pharynx, sc - separated cells, sep - skin epithelium, sp - space beneath skin epithelium.

Staining: Boehmer hematoxylin - eosin. a - ob. 40x, ok. 10x; b - ob. 100x, ok. 10x

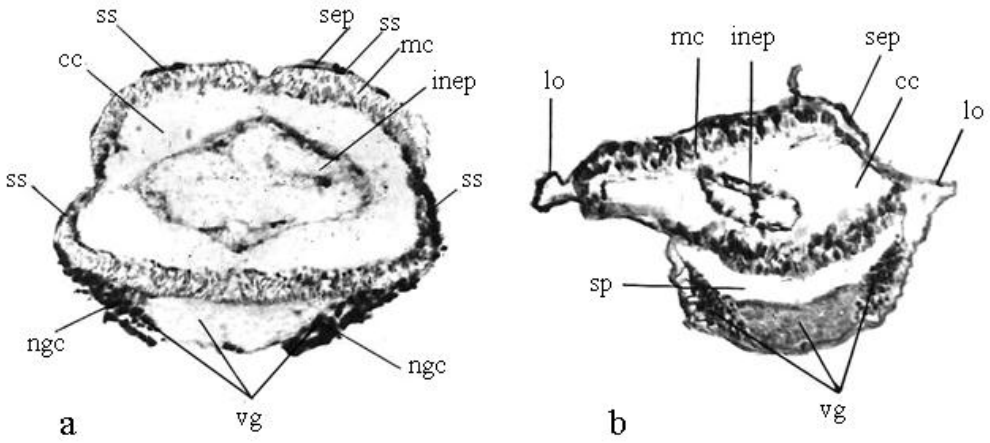

Figure 4. Histological sections of chaetognatha under normal conditions (a) and after radiation exposure (b).

a - transverse section of *Aidanosagitta macilenta* at the level of middle gut and ventral ganglion, b - transverse section of *Aidanosagitta* at the same level.
Indications: cc - coelomic cavity, inep - intestinal epithelium, lo - lateral outgrowths of body wall, mc - muscle cord, ngc - nuclei of ganglion cells, sep - skin epithelium, sp - space beneath ventral ganglion, ss - sensory sites, vg - ventral ganglion.

Staining: a - Nissle method, b - Boehmer hematoxylin. a, b - ob. 40x, ok. 10x

Comparison of the animals' morphology in control and experimental groups showed, that normal chaetognaths have an intact body with a prominent turgor (they do not look flaccid on the forceps). Their alveolar tissue forms an even layer, the fins are complete, eyes are located in symmetry, muscle tissue is rigid. Our data also suggest that fixing the samples in 4% formalin does not affect the morphology. Animals that underwent experimental radiation exposure demonstrated damaged skin epithelium (pathological changes in the alveolar tissue layer) and fins, three animals having lost their chitin apparatus. No other external morphological changes were found. With that said, abnormalities of alveolar tissue and fins were found in 100% of the experimental animals (both living and fixed). Chitin apparatus loss is a rare peculiarity observed in few individuals in Chazhma Bay and in the experiment. Viability of the affected animals in the tanks did not differ from that in the control group.

4. DISCUSSION

Various Chaetognatha species from Chazhma Bay present similar deviations from normal morphology, although the degree of these abnormalities may vary between individual animals, probably reflecting the time of exposure to, and distance from the pollution source.

In the experiment using the radioactive soil as a source of ionizing radiation, we have detected morphological abnormalities identical to those observed in the animals from the plankton samplings in Chazhma Bay. However, the proportion of abnormal animals in the Chazhma Bay samples never reached 100%, the level we observed in the water tanks with radioactive soil. About 5-10% of the animals in the natural catches were normal. This may be explained by the methodology of plankton sampling. The Juday net was descended to the bottom, and after that it was pulled up throughout the depth of the water, while the most affected by radioactivity water mass was contained within two meters above the bottom.

It has been reported (Kasatkina 1995), that the chaetognaths in the regions of high geophysical activity preserve their gut completely intact. As judged by the literature, intestinal abnormalities have not been detected in other ecologically compromised regions similar to Peter the Great bay, as well as in other regions of the World Ocean.

Histological picture of abnormal individuals of the chaetognaths is in a full agreement with the visual findings regarding the skin epithelium changes and intestinal defects reported in the animals obtained from the epicenter of radioactive pollution (Kasatkina & Sergeev 2004). Morphological abnormalities found in the chaetognaths from the radioactively contaminated Chazhma Bay have not been noted after exposure to other hazards, such as heavy metals (Kasatkina, Shumilin & Goryachev 1991), ultrasound (Kasatkina & Karpenko 2001), and geophysical factors (Kasatkina & Stolyarova 2010, 2016), which suggests that the changes observed in this study are specific for radioactive exposure. And, therefore, the morphological changes in the chaetognaths described above may serve as a strong evidence of a radioactive contamination of waters.

Chorology of the chaetognaths is outstanding. They inhabit waters from seashore shallows to deep open spaces, and are found everywhere from the surface to the abyssal. Their representatives can be found in the sea any time of the day, and any time of the year. The transparency of their bodies allows for a visual monitoring of their organs and tissues. Their wide distribution and availability in combination with the simplicity of morphological evaluation of changes in response to external factors suggest that these animals may be used as biological indicators of pollution and, probably, of natural fluctuations in the marine medium, as well.

5. CONCLUSION

Histological findings provided above most markedly revealed those abnormalities that can be seen visually under a magnifying lens or a microscope. New detailed data have been obtained on the changes at the tissue level. These observations allow us to suggest, that ionizing radiation affects a significant part of the chaetognaths' tissues, causing their damage distinct from that seen in the

animals inhabiting areas with enhanced geophysical activity. The major deviations detected are as follows: weakening of connections between cells (with separation of cells from the surface of the skin epithelium), and between adjacent tissues (dissociation of muscle from the skin epithelium). It is possible, that because the muscle tissue do not undergo lysis (in contrast to animals from regions with high geophysical activity) and due to sharp muscle contraction, the separation of the muscular tissue from the epidermis takes place, being the trigger for the detachment of the ventral nerve ganglion. Histological evaluation of these abnormal animals revealed several peculiarities characteristic only for the individuals exposed to radiation in the polluted waters of Chazhma Bay. Nevertheless, in spite of severe changes and abnormalities found in the chaetognaths' anatomy, the animals remain viable, maintain their life cycle, and perform all their functions including feeding and reproduction. In cases when radiation destroys intestinal epithelium, the chaetognaths still remain alive. This is most likely due to their ability to feed on a soluble organic matter (Sorokin & Wyskwarzev 1973). Survivability under various adverse environmental conditions may be explained by the unique organization of these animals. The chaetognaths are characterized by an archaic, primitive organization combined with a highly specialized tissues (Stolyarova & Kasatkina 2009). This histological peculiarity nominates the chaetognaths as candidates for convenient biomarkers of environmental pollution in the sea, as well as indicators of geophysical activity (Kasatkina & Stolyarova 2016). Results of our histological analysis support this role of the chaetognaths. Simplicity of the detection methods makes these animals particularly suited for this purpose.

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