

MARINE ANIMALS *CHAETOGNATHA* AS BIO-INDICATORS OF GEOPHYSICAL ACTIVITY

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Abstract

A study was done on the morphology of marine planktonic animals, Chaetognatha (arrow worms), taken during sea expeditions from the regions without the influence of geophysical activity, from shallow-water volcanic bays and deep-water geophysically active regions. It was discovered that among the chaetognaths from geophysically active regions there was a large number of animals (up to 95%) with morphological anomalies, while the animals from regions unaffected by geophysical activity had no structural abnormalities. Morphological deviations were investigated at the anatomical level using a hand magnifying glass and under a microscope, histological changes were also studied. There have been identified specific tissue anomalies which are not encountered under any other damaging effects of the environment. It is assumed that the observed anomalies of structure occur as a result of lysis of the muscle tissue under the influence of geophysical factors. Chaetognatha are characterized by high viability: anomalous specimens do not die and continue their livelihoods, so they can serve as biological indicators of increased geophysical activity in the region.

Key words: chaetognatha, morphology, histology, ecology

1. INTRODUCTION

Among the most dangerous for human health are phenomena of the geophysical (seismic) activity causing earthquakes and global catastrophes. The harbingers of earthquakes have been established to be changes of composition of atmosphere, the appearance of acoustic noises, a change of magnetic and electric fields of the periearth space, etc. It is known that unusual behaviour of animals both on land and in ocean is one of harbingers of approaching catastrophes. In this connection the study of the state of the biota of ecosystem affected by geophysical activity is one of important components of the problem of ecological safety. Control of ecological situation is necessary for detection of factors harmful and dangerous for human health.

Of particular interest for study of the state of marine biota are Chaetognatha (arrow worms) - the free living marine animals inhabiting waters with a salinity of at least 8 ‰. These animals belong to characteristic and mass representatives of plankton (Kasatkina 1982, Pierrot-Bults & Nair 1991). They have a large chorological range by inhabiting everywhere from coastal shallow waters to open waters, from surface to abyssal waters of all oceans and seas at any latitude. Chaetognaths are planktobenthic animals, i. e., they are able both to freely move in the water thickness and (at certain periods of life cycle) to be located near the bottom (Molchanov 1907, Kasatkina 1971, 1982). Sizes of mature specimens range from 5 to 120 mm (Kapp 1991). Chaetognaths are elongated animals of the arrow-like shape (Stabber 1769). Their body is subdivided into three divisions: the head, trunk, and tail located behind the anus. Present in the upper part of the head are the cerebral ganglion (brain), grasping spines, and teeth. At the dorsal side of the head, behind the brain, there are paired eyes, while at the ventral side there is oral orifice (mouth). At the sides of the body paired fins are located, at the end - unpaired tail fin. The surface is covered by stratified epithelium of unique ultrastructure (Stolyarova and Kasatkina 1988, 2001). Most species of Chaetognatha have skin epithelium thickenings called the alveolar tissue; it can cover certain sites of the body. The muscular tissue is developed in all three body parts, whereas in the trunk and tail it forms four longitudinal cords adjacent to the skin epithelium (Kasatkina 1982). This group is characterized by highly differentiated

peculiar tissues in combination with simple general organization. In cross sections of chaetognaths' body one can see muscle cords, intestine and gonads (Fig. 1). Representatives of Chaetognatha are completely transparent, which provides easiness of their study.

As long ago as prior to the 1990s there were repeatedly noted cases of the appearance of abnormal chaetognaths with visible morphological deviations. Sometimes the abnormalities of chaetognaths were unexplainable. Thus, in the Pacific Ocean (the Japan Trench) from the horizon of 2000-1000 m there were taken two anomalous animals (*Bathysagitta scaphicephala*) (Kasatkina 2001). In the open part of the Sea of Japan in 1968 there was detected a specimen with the caudal fin at the place of the tail. The only explanation was suggested to an abnormal specimen of Chaetognatha without head caught at coasts of Antarctica. Planktonologists (Qresland & Pleijel 1991) under laboratory conditions followed attack of the predator polychaeta *Tiphloscolex* sp. onto the *Eukronia hamata*, in which as a result the head fell away. Occasional morphological abnormalities could have been explained in such a way. There were cases of death of chaetognaths in the bays around Japan that received an explanation: from bacterial diseases (Nagasawa & Nemoto, 1985; Nagasawa, 1986).

However, from the early 1990s, planktonologists have discovered mass appearance of unusual forms of abnormalities among several animal groups. One of the suggestions for the cause of mass appearance of abnormal animals is mechanical damage from screws of numerous ships. However, the screw part even of the motor boat exceeds the length of some abnormal plankters. Besides, subsequent studies showed the highest number of abnormal animals in bathypelagic species whose uppermost habitation border being 200 m. Some planktonologists believe the mass appearance of abnormal chaetognaths can result from damage of animals with net. But in this case it is unclear why they were not revealed prior to 1991 in the samples that were collected by the same procedure from the same sea areas by numerous expeditions beginning from the end of the 19th century. By other suggestions, abnormalities arose as a result of mass attacks of predator polychaetes or crustaceans on chaetognaths or were caused of internal parasites. The emergence of such a strange mass phenomenon in vast regions of the Far East seas stimulated its speedy investigation.

The goal of the present work was elucidation of causes of mass abnormalities in chaetognaths. The research included: collection of material, study of morphology of living and formalin-fixed abnormal specimens, study of abnormal animals on histological sections. Besides, there were performed laboratory observations on normal and abnormal animals and investigation of the distribution of abnormalities in the studied regions.

2. MATERIALS AND METHODS

Materials of this study comprised various species of chaetognaths obtained at different years during research expeditions as well as the species collected near the shore in the Amur Bay during 1968-2006. Apart from Chaetognatha there were studied Tunicata obtained from the same samples.

During the 24th trip of the research ship (RS) "Academician Nesmeyanov" in July-August 1993, the standard plankton collection was performed in the Bering and Okhotsk Seas as well as in adjacent waters of the Pacific Ocean (185 stations, 282 samples). The plankton samples were collected with the JOM net (mouth area 0.5 sq m, No.38 nylon mesh) and Juday net (mouth area 0.1 sq m, No.38 nylon mesh). The maximum depths of the investigated horizons were up to 500 m, in the coastal waters of the lesser depth the samples were collected at each horizon beginning from the bottom by vertical catches. At each station, the salinity, temperature, and the content of oxygen and biogens were measured.

For biocenological studies, plankton was fixed with formaldehyde; for laboratory observations, the living plankton was taken. During the expedition, living animals were observed and abnormal morphological deviations were described. Morphological deviations at the anatomical level were determined with the aid of a hand magnifying glass or the microscope MBS-10, the animals from the sample being placed into the Koch dish for study. There were performed laboratory observations on behaviour of abnormal and normal animals and on the comparative life-span. Under aquarium

conditions it was studied whether the abnormalities were the result of attacks of predator polychaetes, crustaceans or were caused by internal parasites. Distribution of mass findings of abnormal animals in the surveyed regions was studied.

For study of chorology and for investigation of dynamics of the number of normal and abnormal chaetognaths in various years, there were also used plankton collections from 1968 to 2008 in the Far East seas and in adjacent waters of the Pacific Ocean. Important are plankton samples in the Kraternaya Bay of the acting volcano in the Ushishir Island, which were collected by O.G. Kussakin in 1993 (during expedition of RS "Academician Oparin": 7 samples) and by V.G. Tarasov in 2005. For the comparison of morphological abnormalities, there were processed plankton samples from geophysically active volcanic bays in the south-west Pacific Ocean (RS "Academician Nesmeyanov" 1990). These samples present a particular interest, as the animals inhabited this area under conditions of the recorded geophysical activity (Tarasov 1994, 1999). There also were used materials of standard plankton collection in the Sea of Japan: the trip of RS "Professor Levanidov", 1989-1998, of the Far East Research Institute of Fishery and Oceanography (TINRO), and the Far East Hydrometeorological Research Institute: the 56th trip of RS "Ocean", 1994 (8 stations, 46 samples).

The plankton collections taken by hydrologists of Academician V.I. Ilyichev Pacific Oceanological Institute (the Far East Branch of the Russian Academy of Sciences) are important for understanding of processes of transfer of abnormal animals by currents (Supervisor V.B. Lobanov); during the plankton catchings there were the simultaneously carried out hydrological investigations that were compared with satellite data. The most large-scale trips in the Sea of Japan were performed by the RS "Academician Lavrentiev", 1995; "Lugovoe", 1999. Besides, the plankton samples were taken in 1968-2006 from a boat or in winter through the holes in the ice near the shore of the Amur Bay by the vertical catching from the bottom to the surface. These collections were performed by the Juday net (mouth area 0.1 sq m, No.38 nylon mesh).

As a result of numerous expeditions, both urbanized and remote from the anthropogenic influences regions were studied. There were examined the regions without the geophysical activity as well as shallow bays with volcanic activity and the deep-water regions with established geophysical activity. Over 100 000 samples of chaetognaths were analyzed. Ratio between abnormal and healthy plankters in the sample was studied. For the comparison of the data the number of animals was recalculated by the currently accepted procedure (Kotori 1976) in specimens /1 000 m³. Morphological deviations in various plankton animals were visually examined with the aid of a hand magnifying glass and under microscope. For histological purposes, the material fixed with 4% formalin was embedded in paraffin. The 5-7 micrometer thick sections were stained with Boehmer or Mayer hematoxylin and eosin. Histological preparations were examined using a light microscope Leica DME (Leica, Germany), images were taken with a digital camera Leica EC3 (Leica, Germany).

3. RESULTS

3.1. Morphology of plankton animals taken from different regions

Examination of regions that have no geophysical activity did not reveal deviations in the structure of Chaetognatha. Study of the species composition of plankton in geophysically active regions has shown the presence of morphological abnormalities in plankters, mainly among Chaetognatha and Tunicata (Kassatkina, Lapshina & Selina 1993, Lapshina 1993, Kasatkina 1995). Sometimes the deviations are present in Crustacea. In samples from the regions with increased geophysical activity (Eastern Sakhalin, Aleutian Trench) there was noted the highest number of abnormal Chaetognatha (95% of the total number) and of Tunicata (100% of the total number). In the same samples there was studied phytoplankton by the specialist in this area M.S. Selina (Institute of Marine Biology, Vladivostok); however, no abnormalities in the examined groups of phytoplankton were detected. Morphological abnormalities also occur in Copepoda. According to the conclusion of the specialist on plankton crustaceans M.V. Geptner (Zoological Museum, Moscow State University, City of Moscow), abnormality in Copepoda *Preuchcuta elongata* Esterly, 1913, consists in the lesion of muscles, the deviation being present only in occasional specimens (personal communication). This lesion is present

much more seldom in Copepoda than in Chaetognatha and in Tunicata, probably owing to their protection with chitin cover.

3.2. Characteristics of morphological deviations in Chaetognatha from regions with geophysical activity at histological level

On transverse histological sections through the body of abnormal Chaetognatha from the shelf region of the Laptev Sea with a high level of geophysical activity changes in the muscle tissue are present (Fig. 2a). Between muscle bundles forming muscle cords, numerous spaces are observed, whereas in norm, muscle bundles meet closely. Other structures have no visible changes.

On transverse histological sections through the body of abnormal Chaetognatha from the region of increased geophysical activity (Aleutian Trench, the south-west Bering Sea) there are observed sharp deformations and disturbance of symmetry of the body: dorsal muscular cords are significantly shifted in the horizontal plane relative to ventral cords (Fig. 2b). Between muscle bundles in muscle cords there are visible significant spaces. It is also seen that the animal body is compressed in the dorso-ventral direction. Both at the dorsal and at the ventral side, under the layer of muscle there are seen the spaces that are dilated at the level of lateral fields. At both sides of the body, in the areas of lateral fields, dilatations of irregular shape are observed. The observed spaces and dilatations probably correspond to the areas where the dissolution of muscle occurred. Borders of the gut and intestinal epithelium are not identified, in the place of the gut in the centre of the body there is present a mass that does not have a certain structure. The gut also seems to be subject to changes.

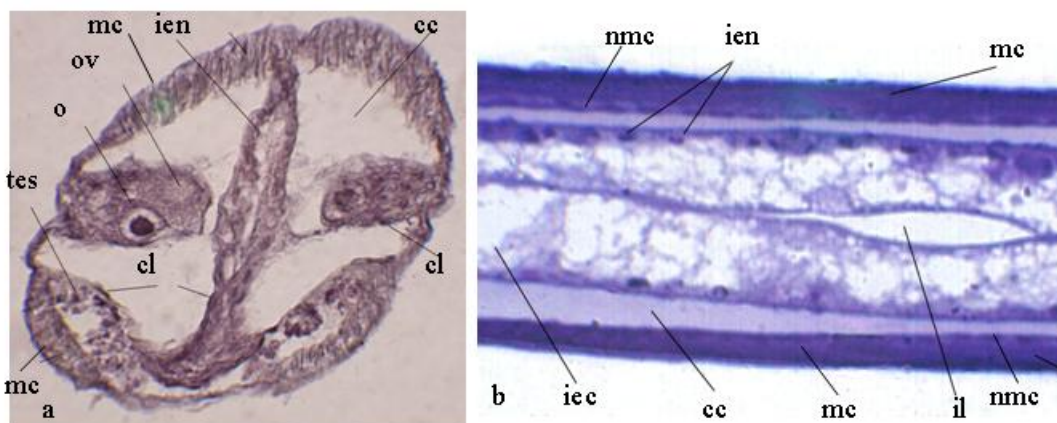


Figure 1. Normal composition of representative of Chaetognatha *Aidanosagitta macilenta*.

a – transverse section at the level of posterior gut (IV stage of maturity); b – portion of sagittal section of middle part of the body (0 stage of maturity).

Indications: cc – coelomic cavity, cl – coelomic lining, iec – cytoplasm of cells of intestinal epithelium, ien – nuclei of cells of intestinal epithelium, il – intestinal lumen, mc – muscle cords, nmc – nuclei of cells of muscle cord, o – oocyte, ov – ovary, tes – testes.

a – paraffin section, staining: Boehmer hematoxylin – eosin; b – semithin (araldite) section, staining: toluidin blue. Ob. 40x, ok. 10x

Comparison of the animals from different regions characterized by geophysical activity shows that in them similar disturbances of integrity of the muscle tissue are present. The observed pictures can be explained by a suggestion about lysis of muscles. These data indicate that the muscle tissue is a main

target of influence of geophysical factors, its changes can lead to the changes of the body configuration.

The described disturbances differ from the changes revealed in Chaetognatha effected by radiation characterized by damaged alveolar tissue and fins (Stolyarova & Kasatkina 2012).

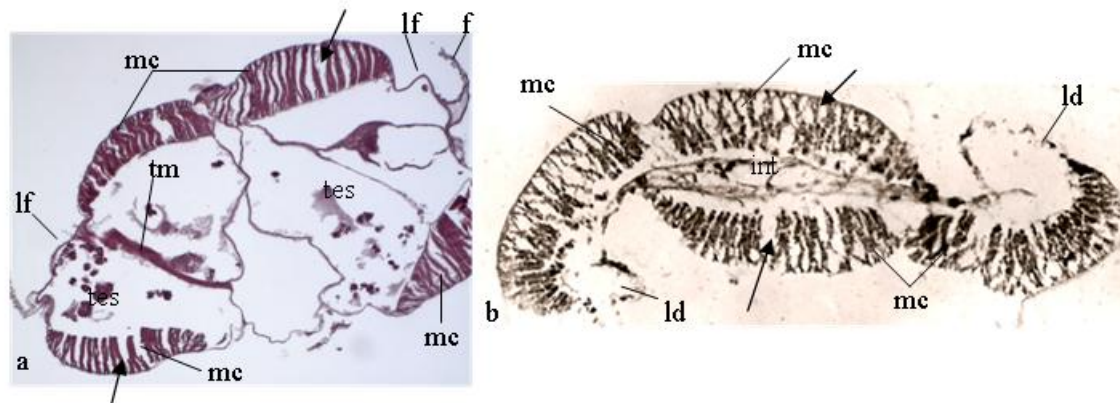


Figure 2. Abnormal Chaetognatha from Laptev Sea (a) and from Aleutian Trench, the south-west Bering Sea (b).

a - cross section of the posterior portion of the body behind the gut of *Protoheterokrohnia* (II stage of maturity), b - cross section of the posterior portion of the body at the level of the gut of *Eucrohnia*.

Indications: f - fin, int - intestine, ld - lateral dilatations, lf - lateral field, mc - muscle cords, tes - testes, tm - transverse muscle. Arrows: spaces between muscle bundles.

Paraffin sections, staining: Mayer hematoxylin – eosin. Ob. 40x, ok. 10x

3.3. Characteristics of morphological deviations in Tunicata from areas with geophysical activity

Among other plankton animals, morphological deviations from norm are detected in Tunicata. The following deviations can be identified.

Light bands or spots. In Tunicata, like in Chaetognatha, there are the most commonly present morphological deviations that look like the lighter spots or bands on the body.

Irregularities of the surface. Many animals have the uneven surface and invaginations on the tail with festoon-like edge.

Separation of tail from the trunk. After dissolution of muscles the covers in Tunicata become turbid and immediately after division of the body into parts the animals die in aquarium.

3.4. Laboratory observations for normal and abnormal animals

Animals were placed into aquaria immediately after catching. Observations on normal and abnormal Chaetognatha specimens were performed in aquaria of a small volume - 1 l, also for the convenience of study under microscope, the specimens were transferred into Koch dishes, whereas others were kept in large tanks (10-30 l), where temperature and salinity were maintained under natural levels.

Survival of abnormal Chaetognatha in aquarium is approximately equal with normal animals - up to two weeks. Interestingly, the abnormal animals keep their life activity - spawn spermatophores from seminal sacs and lay 1-2 eggs.

To check the suggestion that a part of abnormalities in Chaetognatha can be result of a mechanic damage with a net, there were carried out experiments in aquarium with normal animals - a part of the body higher than the ventral ganglion was separated with a scalpel. Healing and scar formation occurred in 24 h. However, tissues were fused differently than in abnormal specimens. This experiment has shown insolvency of the suggestion that abnormal chaetognaths can arise as a result of mechanical damage during catching.

The second question to be answered whether the pronounced abnormalities are result of mass attack of predator polychaetes or crustaceans also got the negative answer. Together with Chaetognatha, the plankton polychaetes from the genus of *Typhloscolex* were placed into aquarium in the amount equal to the number of Chaetognatha. Not a single case of attack of polychaetes on Chaetognatha and vice versa was observed. In spite of that the plankton polychaetes from the genus of *Typhloscolex* were present rather often in samples, the cases of attack neither of polychaetes nor of crustaceans upon Chaetognatha were observed.

A suggestion was checked about effect of parasites on morphology of chaetognaths. In samples of Sakhalin Gulf and in the Sea of Okhotsk in the area of Eastern Sakhalin there were revealed chaetognaths (up to 80% of the total number in the sample) infected with unicellular parasites (Ciliata) detected in coelomic cavity. However, such parasitic infestation did not produce a pattern similar to that observed in abnormal animals. Besides, there were found earlier noted (Nagasawa & Nemoto 1985; Nagasawa 1986, 1991) bacterial skin diseases, as result of which the covers become turbid and shrunken, but the character of morphological changes also has no similarity with that in abnormal animals. Such specimens in aquarium died for several hours.

In Tunicata, in case of lesion of muscle, the animals die in aquarium. In Chaetognatha, abnormal animals not only continue their existence, but also reproduce themselves thus presenting a convenient object for bioindication of powerful, large-scale natural phenomena. For this reason, it is the Chaetognatha, as compared to other plankton organisms, which are a convenient object for ecological monitoring of environment.

3.5. Investigation of distribution of abnormalities in the studied regions

Investigation of distribution of abnormal Chaetognatha shows that spreading of this phenomenon has a wide scale: from the Bering Sea to the Sea of Japan. Since 1986, TINRO performs standard plankton collection in this area; however, since 1991, abnormalities in plankton have been detected in mass amounts. The most significant number (both absolute and relative) of abnormal animals is present not at superficial horizons, but at the depth of at least 500 m. At this depth the highest amount of abnormalities is present in species from the family of Eukrohniidae represented predominantly by bathypelagic species. The number of abnormal Eukrohniidae is sharply reduced with a decrease of depth. This is obvious to be due to the fact that the source causing abnormalities in plankton of the Bering Sea most likely is at the large depth; it is also possible that connected with this is the absence of abnormalities in phytoplankton whose vertical migrations do not have such pronounced range as the zooplankton: copepods, tunicates, and chaetognaths (Kiselev 1980).

There are much less abnormal animals among species from the family of Sagittidae, and the interzonal species *Sagitta kussakini* Kassatkina, 1996, has 35 times less abnormalities than species of Eukrohniidae. The number of abnormal Sagittidae in the Bering Sea is sharply reduced not only with decrease of depth above the Aleutian Trench, but also with approach to the shore. For instance, the species *Parasagitta elegance* that in the Bering Sea is located mainly in the shallow water, near the shore (it is seldom can be seen in depths) almost did not suffer: only occasional abnormal animals are present. With advancement of the Kamchatka Current to the south the number and the ratio of abnormal and healthy animals change in favour of healthy specimens.

4. DISCUSSION

It is known (Sorokin & Wyskwarzev 1973, Wyskwarzev & Sorokin 1978) that chaetognaths are able to consume dissolved organic matter. Possibly it is with this that the long life-span of the abnormal animals is connected. For example, in experiments of Ghirardelli (1968), specimens with amputated head survived for one month in aquarium. Noted is the fact that in the Bering Sea, in areas of high number of abnormalities of chaetognaths, they not only did not extinct, but it is in these areas that the density of concentration of chaetognaths relative to other groups of plankton animals - euphasiids, amphipods, and copepods in 1991 (Shuntov et al. 1993) were even higher than in the area where abnormalities were not observed. As it is established (Khristoforova 1989), in the geophysically active Kraternaya Bay, concentration of the soluble organic substance was significantly higher than in the waters surrounding the Ushishir Islands, the number of chaetognaths inside the bay being significantly higher than in the waters from the external side (Kossikhina & Malakhov 1991).

The spreading of abnormal organisms has a large scale. If previously the cardinal readjustments in plankton populations were observed in urbanized zones (Vinogradov & Shushkina 1992, Vinogradov, Shiganov & Khoroshilov 1995), the described novel phenomenon includes large regions remote from human settlements and estuaries of rivers bearing, as a rule, the anthropogenic pollution. There are known morphological deviations in meroplankton, in larvae of benthos animals under influence of anthropogenic factors (Malakhov & Medvedeva 1991; Vaschenko & Zhadan 1995). It can be suggested that the revealed novel mass morphological deviations in plankters is also the result of anthropogenic actions. However, this type of abnormalities is not present in the regions of shelf having anthropogenic pollution with heavy metals (for instance, in the Zolotoi Rog Bay of the Peter the Great Gulf of the Sea of Japan), although the content of heavy metals in plankters from these regions was extremely high (Kasatkina, Shumilin & Goryachev 1991). Up to 1992 these abnormalities were not revealed also in Sakhalin Gulf although level of heavy metals in plankters from this region in 1988 was as high as in the Amur Bay of the Okhotsk Sea (Kassatkina, Lapshina & Selina 1993).

This type of abnormalities was discovered in the Kraternaya Bay of the Yankich Island of the Kuril Ridge. The Kraternaya Bay, remote from anthropogenic sources, is the natural phenomenon exposed to permanent geophysical activity (Gavrilenko, Chertkova & Taran 1991), as it is a flooded crater of the active volcano Ushishir. The detailed diverse studies of the bay were published in the three-volume edition (Tarasov 1991). The lack of the above-described abnormalities in the anthropogenically polluted regions (for instance, Zolotoi Rog Bay, Gornostay Bay of the Peter the Great Gulf of the Sea of Japan) and their detection in the Kraternaya Bay lead to suggestion about the geophysical activity as a factor causing the appearance of peculiar abnormalities in the plankton biota. In the plankton of the Kraternaya Bay there were not detected (possibly due to a long influence of geophysical factors) Tunicata that are present in the samples taken on shelf of the Kuril Islands. The chaetognaths are able not only to survive under such conditions, but also to reproduce themselves in the abnormal state; therefore, the number of chaetognaths achieves high values - at the period of reproduction 360 specimens / m³ and even higher.

Interestingly, in examination of shallow waters of all Far East seas from Chukotka to Posyet in the mass epiplankton species *Parasagitta liturata* the abnormalities were revealed only at one point - in the Kraternaya Bay (52% in samples). The convincing evidence in favour of effect of geophysical factor on the appearance of abnormalities in plankton we see not only in case of Kraternaya Bay, but also in the geophysically active points of the south-eastern parts of the Pacific Ocean (the Plenti Gulf, the Matupi Bay, etc.). The difference between the Northern and Southern calders in terms of taxonomy is enormous. The geologically young (Bondarenko 1991) Kraternaya Bay is penetrated only by epiplankton *Parasagitta liturata* and *Sagitta kussakini*, widely spread in Far East seas, and is not inhabited by the meso- and bathypelagic species, whereas the volcanic bays of the Southern part of the Pacific Ocean (Matupi, Plenti) are inhabited only by the characteristic of them species of Chaetognatha. In spite of the difference in taxonomy of Chaetognatha between the north-western and the south-western parts of the Pacific Ocean, the above-mentioned morphological abnormalities are present in both regions of the Ocean. Hence it can be suggested that in the rest of the World Ocean the

appearance in the recent years (beginning from 1991) of abnormal phenomena in plankton is related to the awakening of geophysical activity.

Since the abnormalities are not associated with mechanical damages, attacks of other animals or bacterial diseases, there remains the only suggestion - the appearance of significant outbreaks of unusual abnormalities (differing from abnormalities caused by radiation and other factors) among plankters on vast spaces of Far East seas and in the waters of the Pacific Ocean is obviously related to the increase of geophysical activity of Earth.

5. CONCLUSION

Thus, the mass appearance of abnormal specimens in plankton on the vast spaces of Far East seas and in the north-west Pacific Ocean is not a result of mechanical damage or of parasitism, either external and internal. This is a novel anomalous mass phenomenon in plankton biota. This phenomenon is not connected with anthropogenic pollution, involves vast regions often remote from human settlements and estuaries of rivers carrying pollution. By the character of distribution of abnormalities in the ocean and their high number in geophysically active bays it can be concluded that the main cause of their appearance in vast regions is an increase of geophysical activity in the north-west Pacific Ocean and its peripheral seas.

In this connection, it can be suggested that the plankton anomalies possibly may be used to forecast an increase of geophysical activity in fractures of the marine medium.

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