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# Ecological risk assessment to populations of small benthic Crustaceans

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## Abstract

Small benthic crustaceans are considered in comparison to large ones as the most vulnerable in relation to injurious effects according to proposed by us criteria of "critical" populations (species) identification. Experimental cytogenetic data on enfluence of different mutagene equivalent doses of ionising radiation and chemical mutagens (i.e. doses inducing equal mean number of cells with chromosome aberration in %) on *Gammarus olivii* and *Idothea baltica* posterity are analized and compared with results of cytogenetic analysis of natural crustacian populations. For the assessment of specimens reproductive contribution and effective population size (i.e. number of specimens determing genetic structure of next posterities) a number of embryos with spontaneous chromosome mutagenesis (up to 2% of cells with chromosome aberrations) is used as a criterion of postery of full value. It is concluded that cytogenetic analysis of female posterity allows to assess expected reduction of population adaptive possibiliity and ecological risk.

**Key words:** Ionising radiation, chemical mutagens, benthic crustaceans, ecological risk.

## Introduction

Approaches to ecological risk assessment are based mainly on "dose (or concentration) – effect" dependencies (Jones et al., 2003). In our opinion the most constructive and adequate approach to ecological risk assessment is the study of adaptive potential of natural populations first

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at all "critical" species that are very vulnerable to injurious effects. Lost of them may lead to communities degradation.

Some criteria in relation to reference organisms choice were proposed for the purposes of protection of biota against ionising radiation (Pentreath, 1998; Strand et al., 2000) : 1) organisms which, by virtue of environmental transfer and concentration factors, have the greatest potential for exposure; 2) organisms which have a high radiosensitivity; 3) organisms which are important for the healthy functioning of the community or ecosystems; 4) organisms which are common; 5) organisms which have pecularities of radionuclides incorporation.

However, since species consist of populations, so namely populations have to be the objects of protection. We think that the identification of "critical" populations (species) must be based on species and populations characteristics that form population adaptive potential (Tsytsugina, Polikarpov, 2005b).

The aim of this paper is: 1) to show that populations of small benthic crustaceans are the most vulnerable to injurious effects and 2) to assess expected reduction of their adaptive possibilities (i.e. ecological risk) at different levels of deleterious factors influence.

## Material and methods

We have generalized and analyzed our experimental data on action of ionising radiation and chemical mutagens on embryos of *Gammarus olivii* Milne – Edw. and *Idothea balthica* Pallas (Polikarpov, Tsytsugina, 1993, 1996; Tsytsugina, 1998). Females with developing embryos in their marsupial sacks were irradiated in our experiments with help of the γ - irradiator "Investigator" ($^{137}$Cs, dose rate 0.048 Gy/s) or were incubated in water with added radionuclides and chemical mutagens which were created difterent doses or concentrations. Experiments were carried out in summer (June-July), when small females characterized by low fecundity breed and in winter (February) and in spring (March-April). In winter and in spring big females with high fecundity breed. Material was fixed in a 3:1 mixture of ethil alcohol and glacial acetic acid. Embryos were stained with 1% aceto-orceine and squashed in 60% lactic acid for cytogenetic analysis. Chromosome aberrations were scored at anaphase-telophase stage of mitosis. 3–6 embryos from every 5–6 females were studied in every variant.

**Results and discussion**

Figure 1 presents species and populations characteristics proposed by us for the identification of "critical" populations (species) of aquatic organisms. The most important characteristic is the reproductive population strategy, in particular, the reproduction system. We have compared the adaptation rate of aquatic crustaceans populations with such of worms, which have different reproduction modes in the region polluted with sewage and in the Chernobyl zone (Tsytsugina, Polikarpov, 2005a). For several years the frequencies of chromosome aberrations in cells of *Gammarus olivii* and *G.lacustris* embryos (gonochoristic species), in germ cells of *Plagiostomum sp.* (hermaphrodite with cross-fertilization) and in somatic cells of *Stylaria lacustris* with prevalent asexual reproduction (paratomic) have been studied. It is found that studied species with sexual reproduction have higher rate of adaptation to anthropogenic pollution in comparison with species characterized with prevalent asexual reproduction.

Fecundity plays also a great role. Low fecundity together with high sensitivity of germ cells and embryonic stages as well as with long duration of gametogenesis (which increases absorbed radiation doses and pollutants concentrations) may act on dispersion of reproductive contribution of specimens. Increasing of reproductive contribution dispersion leads to decreasing of effective population size (i.e. of number of specimens which determine genetic structure of next posterities) as well as to reduction of genetic variability and adaptive possibility (Crow, Kimura, 1970).

Fluctuation of population size and sex ratio influence upon the effective population size as well. Reduction of population size may be the result of hydrobionts death on all ontogenetic stages (if they have high sensitivity to injurious effects) as well as if there are critical periods in their population life.

We found that pecularities of Amphipoda generation structure create prerequisites for acceleration of microevolutionary processes in populations and contribute to their adaptation to concrete conditions of inhabitance. But the spring period of mass reproduction can be considered as the critical one for populations because the posterity appearing at that time, on one hand, determines the destiny of the populations and, on the other hand, is most sensitive to deleterious factors in the environment (Tsytsugina, Polikarpov, 1998).

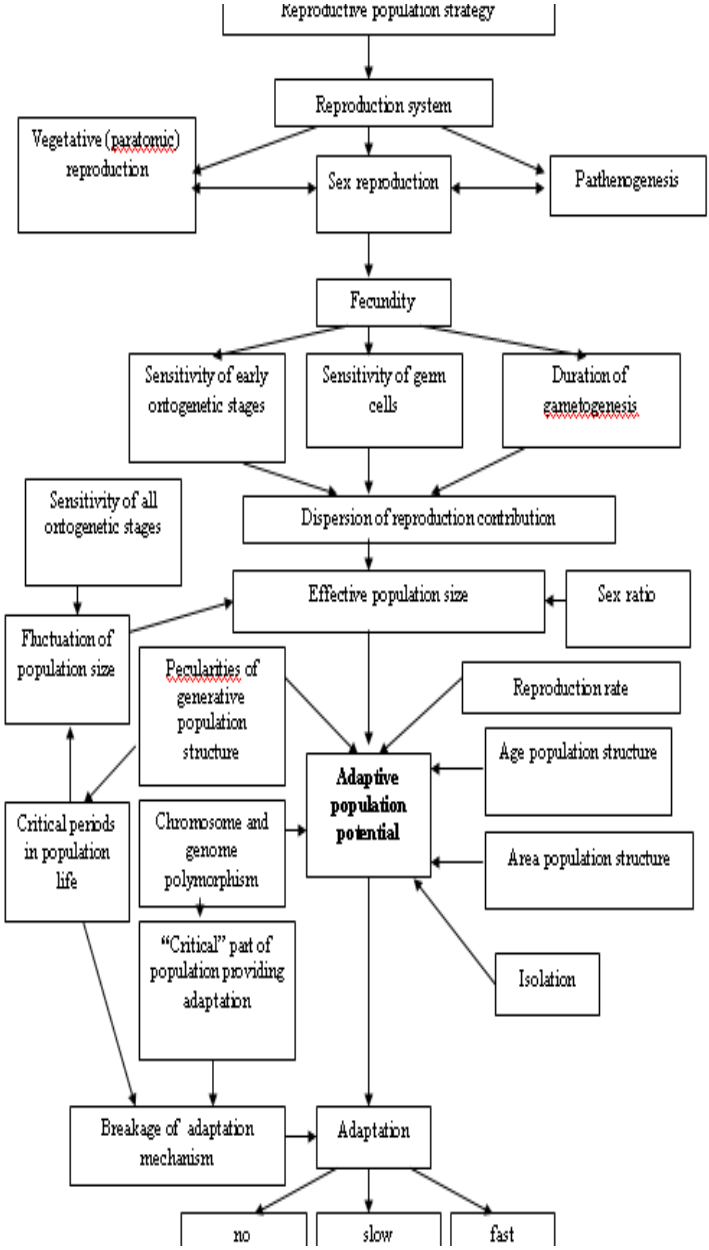


**Figure 1.** Species and population characteristics for the identification of "critical" species (Tsytsugina, Polikarpov, 2005b).

It is known that chromosome and genome polymorphism can also increase adaptive population potential. But in this case the breakage of adaptation mechanism may happen as well. One of us has described chromosome polymorphism in worms *Plagiostomum sp.* population (Tsytsugina, 1992). As the addition to 20 chromosomes there were from 1 to 6 small accessory chromosomes in cells of these worms. About 60% of worms in the population had accessory chromosomes. It is also known that accessory chromosomes influence on the process of recombination. As a result genetic variability increases and adaptive possibility increases too (Volobuev, 1978).

But we observed different sensitivity of germ cells of these worms to the action of $^{90}Sr$ - $^{90}Y$ irradiation (Tsytsugina, Polikarpov, 2005b). Cells with greatest number of accessory chromosomes have higher radiosensitivity. It is shown (Chubareva, 1985) that accessory chromosomes are distributed in germ cells unequaly and deliver great material for the natural selection. It is possible that specimens with big number of accessory chromosomes in cells provide a reserve fund of these chromosomes in populations. The damage of such the most sensitive part of population may lead to breakage of mechanisms both of genome polymorphism and adaptation.

Reproductive rate, age and area population structures as well as genetic isolation obviously also play a great role in the adaptation process.

On the basis of these characteristics we proposed the following criteria of "critical" populations (species): high sensitivity of germ cells and all stages of ontogenesis, low fecundity, pecularities of population generative structure, low reproductive rate, genetic isolation, benthic biotope (Tsytsugina, Polikarpov, 2005b).

Taking into account of these criteria it may be concluded that small (petty) benthic crustaceans (Amphipoda, Cumacea, Tanaidacea) are the most vulnerable to injurious effects. They are most sensitive on all their stages of ontogenesis to anthropogenic pollution (Patin, 1979). These crustaceans (especially small species) have low fecundity in comparison with other ones (for example, Decapoda). Spring period of mass reproduction of Amphipoda and, perhaps, of Cumacea and Tanaidacea which have similar pecularities of generative population structure (Makkaveeva, 1979), may be considered as "critical" one because of high sensitivity of postery.

For the comparison of experimental data on action of radiation and chemical factors we compared effects in mutagen equivalent doses

which are estimated as a mean number of cells with chromosome aberrations (in %) (Tsytsugina, Polikarpov, 2005c).

For the assessment of specimens reproductive contribution which determines the effective population size we used a number (%) of embryos in a population with spontaneous level of chromosome mutagenesis (up to 2% of cells with chromosome aberrations) (Tsytsugina, 1990) as the criterion of posterity of full value because embryos with higher number of chromosome aberrations are less viable. For example, according to (Romashov, Belyaeva, 1966; Tsytsugina, Polikarpov, 2006a) abnormal embryos of fish have higher number of chromosome aberrations than normal ones.

Proceeding from data on the number (in %) of embryos in the population with spontaneous level of chromosome aberrations and the mean fecundity of females we calculated a number of embryos with spontaneous level of chromosome aberrations per a female for four mutagen equivalent doses (5%, 8%, 10% and 13% - 15% cells with chromosome aberrations) (Table 1).

It can be seen that theoretical reduction of effective population size and potential decreasing of genetic variability may be expected in populations under mutagen equivalent doses 5% - 8% if only small female breed (0,9 and 0,25 embryos of full value per a female). Mutagen equivalent dose 10% cells with chromosome aberrations is critical for the posterity both small and big females.

Obviously, the increase of ecological risk may be expected if there is less than one full value embryo per a female.

Numbers (%) of embryos with spontaneous level of chromosome mutagenesis in experiments (Table 1) were compared with ones in natural crustacean populations studied by us earlier (Tsytsugina, 1979, 1990; Tsytsugina, Polikarpov, 2006b) (Table 2). It can be seen also that different species have approximately equal parts of posterity with spontaneous number of chromosome aberrations at the same mean for populations level of chromosome mutagenesis. This data allow us to calculate mean critical levels of chromosome mutagenesis (less than one full value embryo per a female) for populations of small benthic crustaceans with low fecundity (Figure 2). It is clearly seen, that the lower fecundity the higher ecological risk at the equal for populations level of chromosome mutagenesis.

**Table 1.** Reproductive contribution of crustaceans specimens at different mutagen equivalent doses (Tsytsugina, Polikarpov, 2006a)

| Mutagen | Mutagen equivalent doses (mean number of cells with chromosome aberrations, %) | Species | Mean fecundity of females (number of embryos) | Number of embryos in population with spontaneous mutagenesis, % |
|---|---|---|---|---|
| $^{90}Sr$ | 5 | *Gammarus olivii* | 40 | 19 |
| $^{137}Cs$ | | *Gammarus olivii* | 5 | 20 |
| $Pb^{(2+)}$ | | *Gammarus olivii* | 40 | 28 |
| $Pb^{(2+)}$ | | *Gammarus olivii* | 5 | 18 |
| Chlorphene | | *Gammarus olivii* | 40 | 20 |
| $Pb^{(2+)}$ + chlorphene | | *Gammarus olivii* | 40 | 30 |
| $^{137}Cs$ + chlorphene | | *Gammarus olivii* | 5 | 22 |
| $^{90}Sr$ | | *Idothea baltica* | 20 | 20 |
| | | | | |
| $^{90}Sr$ | 8 | *Gammarus olivii* | 40 | 5 |
| $^{90}Sr$ | | *Gammarus olivii* | 5 | 5 |
| $^{90}Sr$+ $^{137}Cs$+ $Pb^{(2+)}$ + chlorphene | | *Gammarus olivii* | 40 | 5 |
| $^{90}Sr$ | | *Idothea baltica* | 40 | 8 |
| | | | | |
| γ- irradiation | 10 | *Gammarus olivii* | 40 | no |
| γ- irradiation | | *Gammarus olivii* | 5 | no |
| γ- irradiation+ Pb(2+) + chlorphene | | *Gammarus olivii* | 40 | no |
| | | | | |
| $^{90}Sr$ | 13-15 | *Gammarus olivii* | 40 | no |
| $^{90}Sr$ | | *Gammarus olivii* | 5 | no |

**Table 2.** Number of embryos (%) with spontaneous chromosome mutagenesis in natural populations of benthic crustaceans.

| Species | Habitat | Mean number of cells with chromosome aberrations,% | Number of embryos with spontaneous level of chromosome aberrations,% |
|---|---|---|---|
| *Gammarus olivii* | Black Sea | | 76 |
| *Idothea baltica* | Black Sea | | 78 |
| *Idothea metallica* | Atlantic ocean | 1.5 | 82 |
| *Melita palmata* | Aegean Sea | | 85 |
| *Gammarus olivii* | Black Sea, sewage zone | | 44 |
| *Pontogammanus crassus* | Dnieper River, Kakhovsky reservoir | | 40 |
| *Pontogammanus crassus* | Dnieper River,Kiev reservoir | 4 | 45 |
| *Idothea baltica* | Black Sea, sewage zone | | 43 |
| *Pontogammanus crassus* | Dnieper River, Kremenchug reservoir | | 18 |
| *Pontogammanus robustoides* | Dnieper River, Kremenchug reservoir | 6 - 7 | 13 |
| *Pontogammanus robustoides* | Dnieper River, Kakhovsky reservoir | | 11 |
| *Gammarus lacustris* | 10 – km Chernobyl zone | 9 | 3 |


**Figure 2.** Critical levels of chromosome mutagenesis for populations of benthic crustaceans with different fecundity

## Conclusion

On the basis of species and population characteristics proposed by us the small benthic crustaceans are considered as "critical" populations (species). From the presented experimental data on the action of ionising radiation and chemical mutagens on embryos of *Gammarus olivii* and *Idothea baltica* as well as from cytogenetic analysis of natural crustacean

populations it is concluded that cytogenetic analysis of female posterity allow to assess expected reduction of population adaptive possibility and ecological risk.

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