X-ray irradiation of male crayfish to control invasive populations: encouraging but not predictable results.

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X-ray irradiation of male crayfish to control invasive populations: encouraging but not predictable results

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Given that the impact exerted by non-indigenous crayfish species (NICS) is most often severe, can occur across many levels of ecological organization, and results in the loss of native crayfish populations, their control/eradication is felt as an environmental priority by the United Nation Convention on Biological Diversity (CBD) at the 1992 Earth Summit in Rio de Janeiro, but any attempt to manage them has resulted to be a flop. To be precise, none of the several attempts made to contain the spread of invasive crayfish in the last decade has been definitive (reviewed in [1]). It is clear the need of new, innovative and more efficacious techniques for managing invasive crayfish. The Sterile Male Release Technique (SMRT) has been successful in the control of insect pests (e.g. [2]) and it could be potentially applicable also in the case of invasive crayfish.

Sterile Male Release Technique (*SMRT*): a Background

The SMRT is based on sterilizing by X-ray radiations and releasing large numbers of males to mate wild females, who will then produce non-fertilized eggs. This technique is highly species-specific and causes no environmental contamination or non-target impacts, differently from the use of biocides or other chemicals. The treated individuals are not radioactive, so they can handled (or even eaten) in complete security or they can be released in nature without any dangerous for the environment. Moreover, since sterile specimens actively seek a mate, this technique offers the additional advantage to assure an efficacious control also at early stages of colonization with low density populations, when other traditional density-dependent techniques fail (e.g. the trap ability is very low in small populations).

Protocols to sterilize crayfish are already developed for *Procambarus clarkii* [3] and recently improved in order to treat many individuals together, with a containment of costs and time (Fig. 1). Encouraging results are also obtained in managing wild populations, suggesting that the release of a sufficient number of irradiated males can actually decrease the size of the invasive population.

The open question was: How much high is the optimal radiation dose? An optimal radiation dose should be able to guarantee a more efficacious impact on male fertility without either compromising sexual responsiveness and competitive ability of the treated animals.

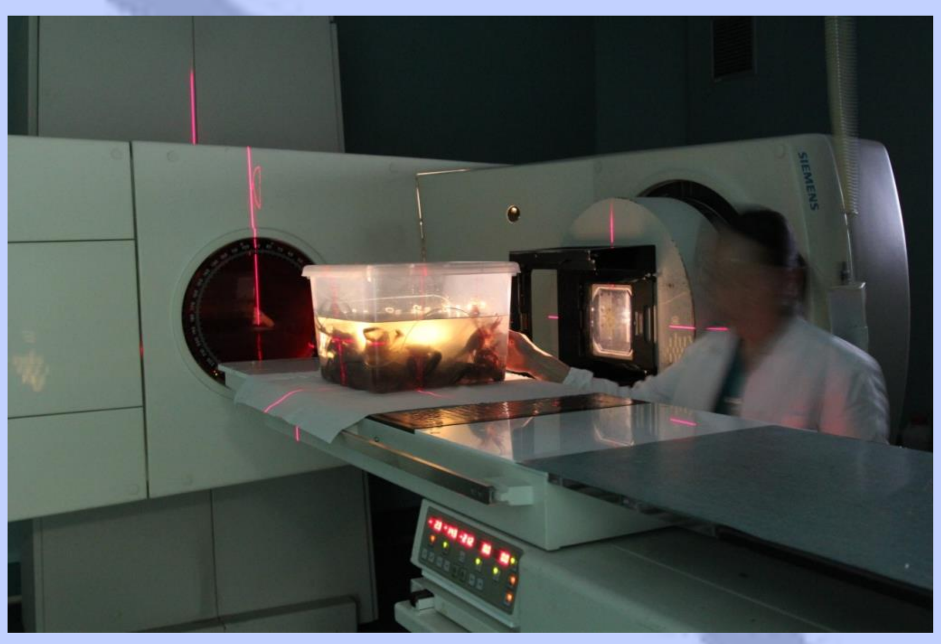

Figure 1. Linear accelerator and maintenance of the crayfish during the irradiation. Animals were treated in water at high densities in tanks covered with Plexiglas.

Aims

Here we tested the effects on gonads, mating behaviour, and reproductive output induced by different X-ray doses to identify the optimal dosage to produce sterile males in the invasive creyfish *Procambarus clarkii.*

Methods

A control group of males (C) was compared with groups of irradiated males at 20, 40, or 60 Gy (n=30 per group). The minimum dose of 20 Gy was chosen in accordance with [3].The irradiation was carried out at the beginning of July at the Careggi Hospital (Florence, Italy). After treatment, males were isolated for two weeks and then paired with a receptive female to assess the effects of irradiation doses on mating behaviour and reproductive output. Afterwards, 5 males from each groups were used for gonad analyses, and additional 5 males (2 C and 1 from each treatments) were used for the quantification of the reached sterility by flow cytofluorimetry.

Results

The irradiated testis shows spermatogenic germ cells with anomalous meiotic division and supporting cells containing up to 3 micronuclei. In the 60 Gy-testis, the seminal acini contain only cells with large vacuolation and membrane rearrangements. The GSI ($F=10.29$, $df=3.3$, $P<0.001$; Tukey test: C>20=40=60) and the size of testis from control is larger than those of the irradiated males (Fig. 2). No behavioural alteration occurs, even if a slight -but not significant- shortening of the copula was recorded with 60 Gy ($F=0.9445$, $df=35.83$, $P>0.05$). Increasing doses produced a progressive reduction of male fertility (Fig. 3), but the flow cytofluorimetry shows the absence of aploid cells already at 40 Gy.

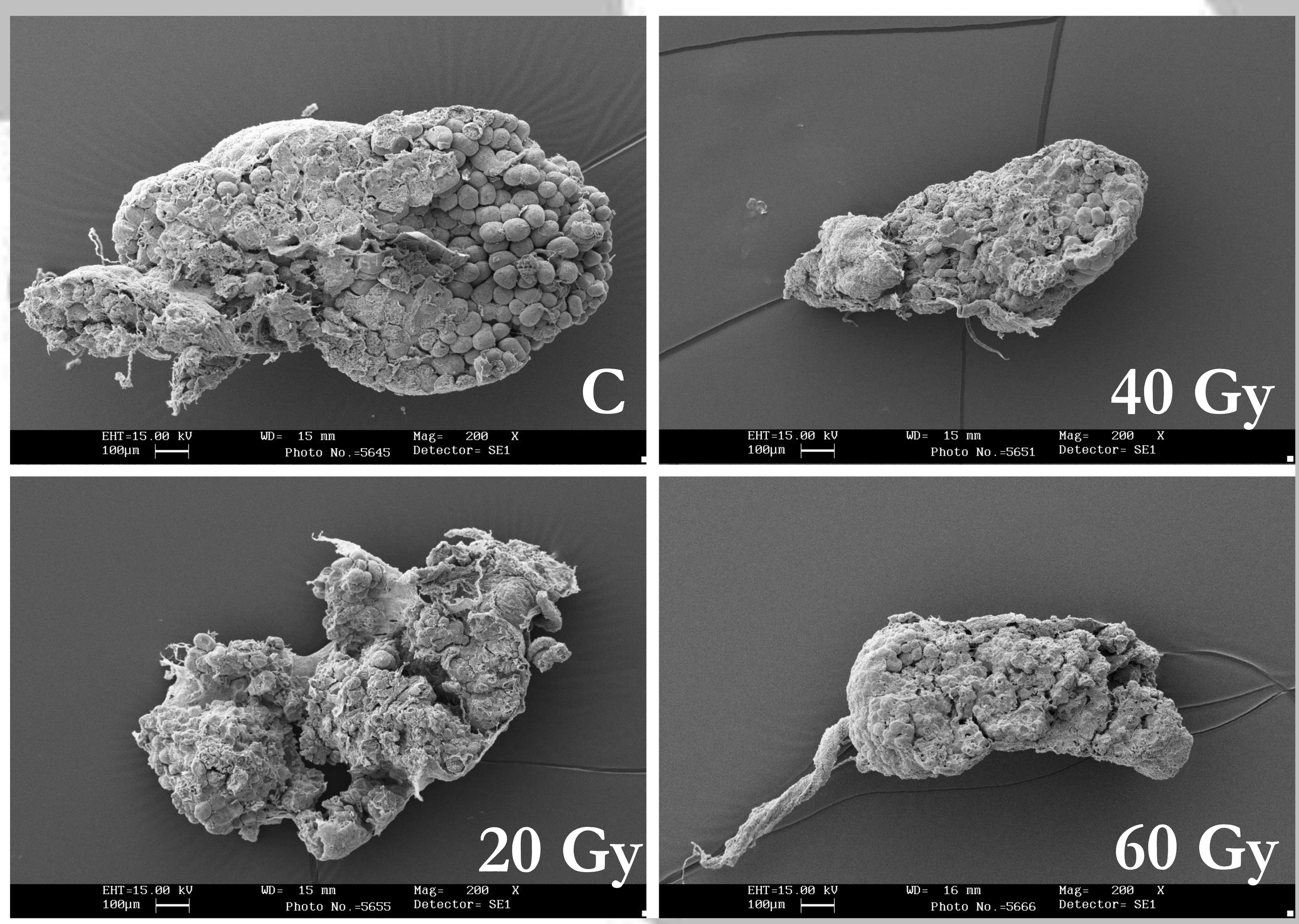

Figure 2. Cross-view of testis observed by scanning electron microscopy of Control and irradiated males at 20, 40 and 60 Gy.

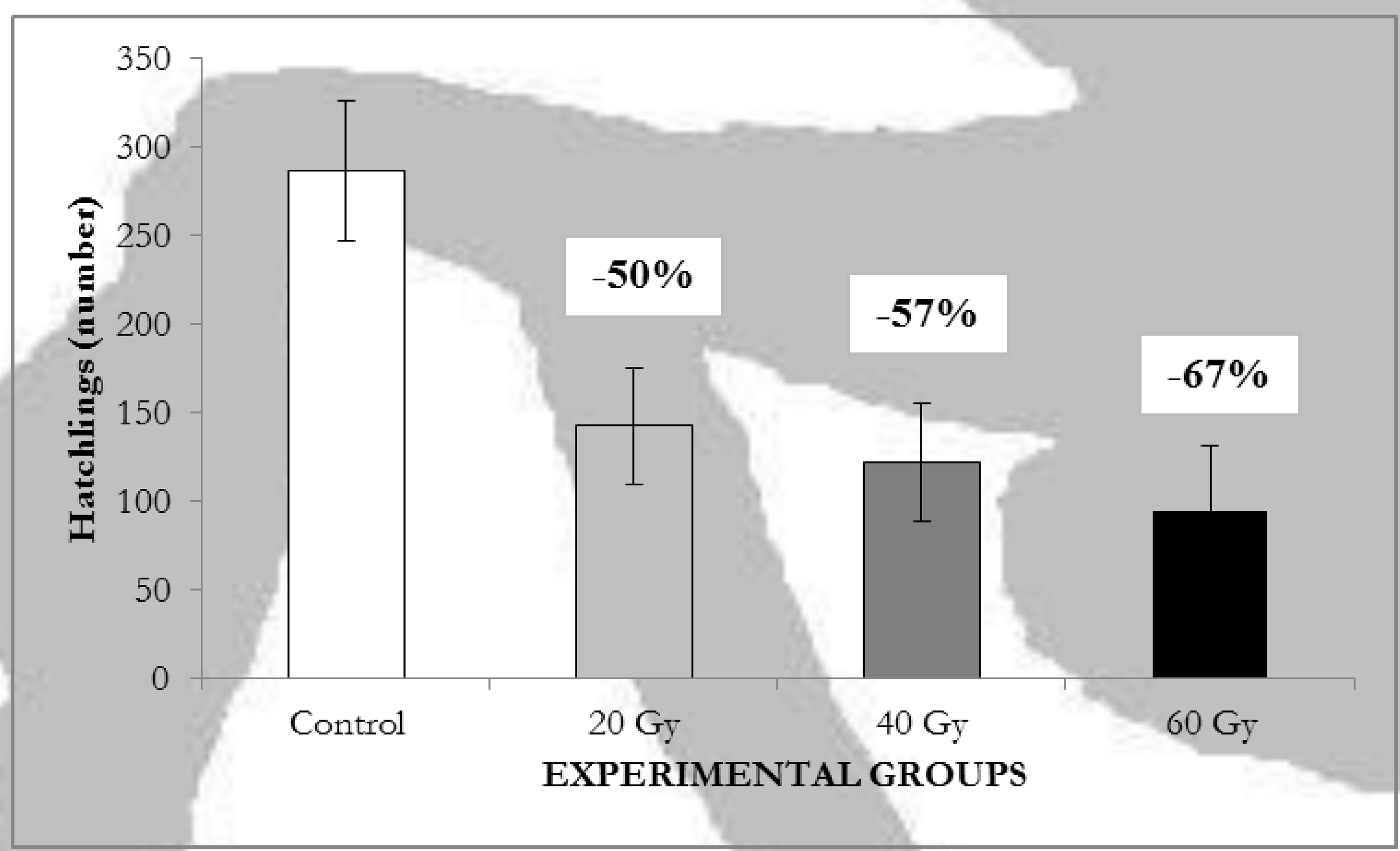

Figure 3. Mean (±SE) of hatchlings of females paired with a Control or irradiated males at 20, 40 and 60 Gy. N=17 per group ($F=4.523$, $df=35.47$, $P<0.01$; Tukey test: C> 20=40=60).

Discussion & Conclusions

The damages recorded in the testes increased with irradiation dose, but the effect obtained at 20 Gy is not comparable than those reported in Aquiloni et al. [3], who firstly described the effects of such a dose in the same species: the damages potentially produced by a X-ray dose are strictly dependent by the biological cycle (i.e. meiotic rate). So, a better identification of the irradiation time is crucial to achieve highest male sterility per dose. Besides, the consistent reduction on male fertility at 40 Gy, together with the absence of behavioural alterations in the mating, could effectively pull down crayfish recruitment. Mathematical modelling will contribute to establish the appropriate number of sterile males to release in relation to the context of application and to the initial population density, but this innovative technique is ready to be applied for managing invasive *P. clarkii* populations.

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Acknowledgments

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