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An easy, non-destructive, probabilistic method to evaluate the imposex response of gastropod populations

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Abstract

Endocrine disruption in marine populations is a growing concern. One of the best documented examples is imposex, the development of male characteristics in female gastropods, including the development of a penis and/or vas deferens, by exposure to organotins, mainly tributyltin. This syndrome has been an effective and widely used biomarker of organic tin contamination for almost 20 years. However, established imposex evaluation protocols sacrifice all the animals and repeated assessments can result in depletion of populations. To preserve animal lives as much as possible and to have a fast screening of areas subjected to organotin impacts, a non-destructive approach was tested. This approach uses reversible narcotization and a statistical criterion to evaluate the extent of imposex in the populations, instead of imposex indexes, which are based on evaluation of sacrificed, fixed animals. The data consist of two categories: males plus masculinized females in one category and normal females in another. The probability is then calculated that this sample could have been drawn from a normal population. This new method was tested in two contrasting areas of Brazil and with two different species, and the results compared to the conventional evaluation. Narcotization was completely reversed with a survival rate of 98%. Impacted sites could be identified with this technique in the same way as with the conventional one. The only limitation was that female sterility could not be verified. This disadvantage could well be compensated by the minimal mortality in the studied populations.

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1. Introduction

Organotin-based paints are widely recognized as the most efficient antifouling system ever used. However, they are highly toxic for non-target species, persistent in the environment, and capable of entering marine food chains, being detected in marine mammals and birds. Their use is controlled in many countries, and a global ban on their use was scheduled by IMO (International Maritime Organization) to start in January, 2003, but the Convention is not yet in force. Reviews on their ecotoxicology (Fent, 1996), environmental behavior (Hoch, 2001), the socio-economic aspects of their application (Champ, 2000) and their effects on marine mammals (Tanabe, 1999) are available.

The endocrine disruption syndrome called imposex, the development of male sexual characters such as a penis and/or vas deferens on female marine gastropods, is caused only by organotin compounds such as tributyltin (TBT) or triphenyltin (TPT) (Smith, 1981; Bryan et al., 1986; Gibbs and Bryan, 1987; Horiguchi et al., 1997; Mensink et al., 2002; Axiak et al., 2003). This syndrome is the clearest case of endocrine disruption by a specific compound in the environment (Matthiesen and Gibbs, 1998). After an initial development phase, when some imposex severity indexes were proposed and tested (Smith, 1981), the basic approach for environmental monitoring with imposex development indexes such as the VDSI (Vas Deferens Sequence Development) were established by the team of Gibbs, Bryan and collaborators (Gibbs and Bryan, 1994, and references therein). This approach has been adapted for use in many species (Fioroni et al., 1991; Stroben et al., 1995; Solé et al., 1998; Ten Hallers-Tjabbes et al., 1994; Horiguchi et al., 1994; Mensink et al., 1997; Mensink et al., 2002), and has been employed successfully in numerous studies around the world. The only drawback of this approach is that the necessary observations of the anatomic details in the interior of the palial cavity of the animals requires this cavity to be opened by cutting through the hypobrachial gland and the gill. To make this dissection, the soft tissues must be removed from the shell by breaking it, killing the animal.

For several years our group has been trying to find a way to evaluate imposex development in gastropod populations without killing them. An alternative approach is the one which would avoid breaking the shells, and also include a narcotization procedure that could be reversed after examination. The first requirement means that observations on the final vas deferens development, vulva blockage and histological examination of the gonads are ruled out as part of the analysis. When properly narcotized, however, the anterior part of their body can be easily pulled out of the shell, and the anterior part of the palial cavity studied. In this way, it is very easy to examine penis and anterior vas-deferens development in both males and females.

To evaluate the degree of imposex in a populations, a statistical approach was developed using the information available without killing the animals, instead of an index that depended on sacrifice and histological examination. This new approach is based on the sex ratios, which are distributed in a binomial form: male or female. There is also in this case a third condition, imposex females. But if we treat these females as ''males'', because of the presence of external male sexual characteristics, we will find some populations formed mainly by males. This condition was previously described in the literature in other