THE IMPOSEX STATUS IN RAPANA VENOSA POPULATION FROM AGIGEA LITTORAL

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Abstract. We investigated the occurrence of imposex in Rapana venosa, an invasive predator on the Black Sea coast. All examined imposex females had unfunctional vas deferens. There were registered no significant differences in the normal females and imposex females sizes.

Keywords: Rapana venosa, imposex, Agigea, gonadal histological sections.


Cuvinte cheie: Rapana venosa, imposex, Agigea, secțiuni histologice ale gonadelor.

Introduction

The large marine gastropod Rapana venosa is an invasive predatory species and its spreading area has been increasing very quickly; last studies have mentioned some native populations in Rio del Plata estuary, on the French coast of the English Canal and on the Holland coast (Harding & Mann, 1999, 2005; Mann et al., 2006).

This species is characterized by a good tolerance and adaptability to varied environmental conditions and developed a reproductive strategy of early mature specimens (young ages and small sizes). Having long living individuals, app. 15 years, a high rate of prolificity (many larval hatchings yearly), a larval planktonic stage (increases its spreading out) and without any special preferences for habitat conditions and nutrition, this species has all the opportunities to spread very quickly in the marine environment.

In aiming at the assessment of Rapana venosa impact on the autochthonous fauna, we continued our study on ecology and biology of this species from the Romanian Black Sea coast and made some observations on sex-ratio (the incidence of imposex in Agigea rapa population), a histological study on gonads for being able to estimate the multiplication coefficient and gonadal synchrony depending on the collecting period.

Material and Methods

In august 2008, we collected 134 individuals from the Agigea 4-5 m depth which were sacrificed in order to provide information on morphology and sex-ratio. There were recorded the shell length, total weight, wet tissue weight, penis length. Every snail was classified as female, imposex female and male using these features: females with no penis had yellow gonads; imposex females had penis (1.14 mm-6.94 mm) and yellow-orange gonads and males with penis (12.26 mm-32.44 mm) and red-brown gonads.

For histological study, there were dissected fragments from anterior, middle and posterior gonads from 16 specimens collected in Julie and August (by 3males, 3 females and 2 imposex females) and preserved in formaldehyde 5%. All samples were dehydrated in alcohol, embedded in paraffin, sectioned at 5μm. Sections were lubricated with Mayer
albumin, fixed on the glass slides, then dried and colored (with hematoxylin), dehydrated in alcohol, washed in benzene and xilen and fixed in Canada balm. There were analyzed a total of 96 permanent histological sections.

**Results and Discussion**

Imposex is known for more than 70 gastropod species (Mann _et al._, 2006), mostly from Muricidae, Buccinidae and Nassidae families (Shi _et al._, 2005). It can be induced by tributyltin TBT and some other organic trialkyl compounds (even concentrations as low as the ng Sn l⁻¹ level) (Bryan _et al._ 1988). Because of these chemical compounds, significant changes can be registered in the gastropod populations. The contamination is possible by water, by sediment (gastropods are hiding in the sand) or by eating contaminated food (bioaccumulation).

There have been published no studies on the occurrence of imposex in _Rapana_ populations from the Black Sea (Chukhchin, 1984; Zolotarev, 1996).

The imposex of rapa whelks from Agigea was characterized using the vas deferens sequence (VDS) index (Gibbs _et al._, 1987; Mann _et al._, 2006). All examined imposex whelks from Agigea were Stage 2 on the VDS scale (the imposex females presented a small penis and vas deferens continuing up to the genital papilla).

From a total of 134 specimens, 51.87% were males, 36.84% were females and 11.19% were imposex females.

![Figure 1](image-url)

**Figure 1.** Numerical distribution rate of sex-ratio rapa whelks (1-males, 2-females, 3-imposex females).

Sex-ratio data indicates a numerical dominance of males and a dominance of females comparing with the imposex females (F:I=3.26:1). Because all imposex females had not functional vas deferens, we expected that this masculinisation of rapa females did not affect the reproductive capacity of individuals.

**Table 1.** Data on number of individuals, sex-ratios index and shell length.

<table>
<thead>
<tr>
<th>Location</th>
<th>Nr. of individuals</th>
<th>Sex-ratio</th>
<th>Shell length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Imposex Females</td>
</tr>
<tr>
<td>Agigea</td>
<td>70</td>
<td>49</td>
<td>15</td>
</tr>
</tbody>
</table>
After microscope analysis of histological sections we noticed the following situations:

**Males**

1. **Gonad in the growing stage (2 specimens)**

   The anterior gonad was characterized by the presence of follicles with immature sexual elements: spermatogonia, spermatocytes and spermatids. The mature gonad from the anterior section is app. 1/3, the immature part is the dominant one with spermatogonia and spermatocytes. The middle and the posterior parts are characterized by the presence of large follicles with spermatocytes (2/3) and the mature part represents 1/3 of it.

![Figure 2](image2.png) Transverse section in the male gonad, anterior section, detail (120×).

![Figure 3](image3.png) Transverse section in the male gonad, middle section, detail (120×).

2. **Mature gonad with sexual elements capable of fertilization (4 specimens)**

   All three gonad sections (anterior, middle and posterior) appear mature, with releasing spermatozoa.

![Figure 4](image4.png) Transverse section in the male gonad, anterior section, detail (300×).

![Figure 5](image5.png) Transverse section in the male gonad, middle section, detail (300×).

**Females**

1. **Gonad with male sexual elements in the anterior part and female in the middle and posterior part (1 specimen)**

   This particular case of female gonad was very similar with the structure of imposex female gonad that will be presented further on. Unlike females, imposex females presented penis and vas deferens.
The anterior gonad was characterized by the presence of follicles with releasing spermatozoa. The middle and the posterior gonad presented ovarian follicles, with mature oocytes, full of yolk granules. Some of oocytes are placed in the centre, and other ones, bigger, are placed on the edge of follicle.

Figure 6. Transverse section in the female gonad, anterior section follicle with spermatozoa (300×).

Figure 7. Transverse section in the female gonad, middle section, follicle with mature oocytes, detail (300×).

2. Mature gonad with sexual elements capable of fertilization (5 specimens)

The initial part presented different shapes of ovarian follicles, some of them small, others bigger, with mature oocytes, full of yolk granules. For these oocytes, diameter varied between 34.5-59.5 µm. The middle part looks similar to the posterior part of the gonad with ovarian follicles, mature oocytes and full of yolk granules, capable for fertilization.

Figure 8. Transverse section in the female gonad, ovarian follicle, detail (120×).

Figure 9. Transverse section in the female gonad, mature oocytes, detail (300×).

Imposex females

Anterior gonad with male sexual elements and middle and posterior gonad with female sexual elements (4 specimens)

The anterior part presented follicles with spermatozoa. The middle and posterior part presented ovarian follicles with mature oocytes. Imposex females presented penis and vas deferens.
Figure 10. Transverse section in the female gonad, anterior gonad, follicle with spermatozoa (120×).

Figure 11. Transverse section in the female gonad, middle gonad follicle with mature oocytes, detail (120×).

Conclusions
We presented in our study the imposex status in *Rapana venosa* females and the sex-ratio for a lot of specimens collected from Agigea 4-5 m depth. Data indicated a numerical dominance of males comparing to females and a dominance of females comparing to imposex females. The imposex expressed by histological sections indicated the anterior gonad with male sexual elements and the middle and posterior gonad with female sexual elements. Hermaphroditism can be considered as a transition between two reproduction phases, because the reproductive capacity of females was not affected.

On behalf of histological gonadal aspect, Julie and August appear to be the most reproductive favorable months for rapa whelks from Romanian Black Sea, when it was noticed a synchronous development, but this study must continue in future.

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References


