The Evidence of Imposex in Turbo sp. from Ujungpiring Waters of Jepara

RAT Nuraini*, R Hartati, H Endrawati, Widianingsih, MJ Rachma, RT Mahendra Jaya

Marine Science Department, Faculty of Fisheries and Marine Science, Diponegoro University Jl. Prof. Soedharto, SH, Tembalang, Semarang 50275

Email: riaazizahtn@yahoo.co.id

Abstract. Imposex is an endocrine disruption syndrome, in which females of marine gastropods develop sexual characteristics of males (penis and/or vas deferens). This syndrome is caused by tributyltin (TBT) or triphenyltin (TPT), toxic organotin compounds found in naval paints used as antifouling system in boats and artificial structures. The main objective of this study was to assess the incidence and severity of imposex in Turbo sp. A hundred individus of Turbo sp was collected from Ujung Piring waters of Jepara, and observed for their sex and imposex. The results showed that the ratio male and female was 1.13 : 1, and the frequency of imposex females was 64.58%. The relative penis size were longer in imposex female than in male. The possibility of pollution was clearly shown from high evidence of imposex female.

Keywords: gastropod, imposex, Turbo sp, Jepara

1. Introduction

A shipping activity in coastal areas is an example of the causes of marine pollution. Generally, The shipping activity happens on the ships which use antifouling paint containing of tributyltin on the body structure and ship propellers. The effects of the use of tributyltinare very hazardous to the incident conditions including macrozoobenthic organisms existence in it.

According to [1] the concentration of tributyltin 1 µg./l itself can lead to the imposex. For example, in 2003 in Jakarta Bay It was found that the concentration of tributyltin in sea air was reaching 15 µg./l and the bladder in sediment attained 649 µg./g. So the imposex level occured in Jakarta Bay is very high.

The regulation in the use of tributyltin was applied in many countries in the late 1980s, which was aimed to reduce the vessels further so, the result of the regulation could bring down the concentration of tributyltin in the estuary water column normally, which was dominated by the small vessels. However, it does not mean concentration reducing of tributyltin was over. The contamination happened previously remained enough high level at some events and the chronic effects of sensitive marine organisms. This is due to the attraction between tributyltin and sediment so it can settle and locate in the sediment. The deposition of tributyltin in the sediments may affect on benthic organisms that can accumulate tributyltin from sediments in the gastropods so anomalies occur in the anatomy of the gastropods [2].
One of the biota affected by tributyltin is gastropods. Tributyltin can lead to the occurrence of imposex on the female and male antelope, that result in the destruction of the reproductive system so it can be able to affect their population. This study is aimed to observe the occurrence of the nozzle on the body of the gastropod Turbo sp. Which is located in Ujung Piring waters which has a shipping activity in Mlonggo Sub-district, Jepara Regency.

2. Material and method
The research material used in this research is gastropod organism typed of Turbo sp. It was obtained from Ujung Piring water, Mlonggo sub-district, Jepara regency. The number of gastropod samples used is 100 pieces, with heights of ranging approximately from 1.2 cm to 2.5 cm. Samples were acquired at each point and then inserted into a plastic zip lock and stored in an ice cool box to keep the sample fresh until it is brought to the laboratory for the further analysis.

Sample of gastropods that have been compiled and cleaned in the laboratory, then placed on the tray for identification. Sampling can be done by viewing at the morphology of the gastropod shell, such as the shape of the shell, the color of the shell, and the shape of the operculum, which is then matched with the gastropod guiding book [3]. The entangled samples in the height of the shell, the width of the shell, and the width of the operculum use a sliding span having a precision of 0.1mm. Once measured, the sample is weighed out by its total weight and the sample of shell is cracked by using a hammer cautiously. The body gastropods from the shell is weighed to get the weight data.

The imposex observation on the gastropod samples were carried out by using petri dishes and the help of loops. The first thing to observe is to know the sex of the gastropod by looking at the color of the gonads. Female sex can be determined based on the presence of a cream or white capsule gland (cg), albland gland (ag) that is dark brown or black, sperm ingesting gland (sig) white and yellow ovary. While the male sex can be ascertained based on the existence of prostate gland (pg) is yellow or brownish orange and testis are yellow or orange. In the male gastropods can be found seminalis vesicle (sv) forming channels resembling a white tube winding [4].

Then the next step is to see the position of the gastropod's tentacle, because the location of the gastropod reproduction is at the lower right part equivalent to the location of the tentacles and close to the gonads [5]. Individual which has the female's imposex, can be inferred to have pseudopenis and or vas deferens at the vaginal opening [1]. While individuals which have the male imposex can be inferred through their small prostate gland and the addition of a large penis to the seminal vesicle [6]. While the ranging height of pseudopenis is the total height of pseudopenis (mm) divided by the number of measurements [1].

The determination of the imposex can be determined by calculating the Relative Penis Size Index (RPSI) according to the manner researched by [7] and [4]. Relative Penis Size Index (RPSI) is used for each population defined as the most of the average female penis formulated as a percentage of most average male penis [8]. RPSI calculation formula as follows:

$$RPSI = \{(Average\ height\ of\ female's\ pseudopenis)^3/(Average\ height\ male's\ penis)^3\} \times 100\%$$

According to [5], the imposex can be seen through the value of the frequency of the imposex (Fi) which can be obtained from the following formula:

$$Fi = (No.\ of\ Imposex\ female/No.\ Total\ female) \times 100\%$$

3. Result and discussion
The result of observation on 100 indivs. Turbo sp. from Ujung Piring Waters, Sekuro Village, Jepara Regency showed that there were three gastropods condition, i.e. normal male (53 indivs.), normal females (16 indivs.) and imposex females (31 indivs.).

The height and width of samples were ranged of 14.35 – 24.7 mm (height) and 13 – 21.6 mm (width), while the total and body weight were 2.12– 8.7 gr and 0.85–2.78 gr. The lenght of male’s penis and female’s pseudopenis were 1.1–8.7 mm and 1.5–7.2 mm. (Table 1).
There was significant different on ratio of shell height of imposex female and normal female (Table 2). The evidence of imposex female *Turbo* sp. was 64.58%. Relative Penis Size Index (RPSI) of *Turbo* sp. From Ujungpiring Waters was 187.86%. The average penis height on normal male and pseudopenis of imposex female was shown in Table 3.

### Table 1. The morphometry of *Turbo* sp. From Ujungpiring waters.

| No. | Status       | Total | Shell height (mm.) | Shell Width (mm.) | Total Weight (gr.) | Body Weight (gr.) | Penis or Pseudopenis Height (mm) |
|-----|--------------|-------|--------------------|-------------------|--------------------|--------------------|----------------------------------|
| 1.  | Male         | 53    | 14.35-24.6         | 12.85-21.6        | 1.74-8.34          | 0.98-2.73          | 1.1-8.7                          |
| 2.  | Female       | 16    | 14-24.7            | 13-21.2           | 2.2-8.7            | 0.85-2.78          | -                                |
| 3.  | Imposex female | 31    | 14.65-22.65       | 13-20.3           | 2.43-6.5           | 1.3-2.25           | 1.5-7.2                          |

### Table 2. Average shell height imposex female and normal female and ratio of imposex female and normal female *Turbo* sp.

| Status of female | Total | Average shell height ± SD | Ratio of imposex female and normal female |
|------------------|-------|---------------------------|------------------------------------------|
| Imposex Female   | 31    | 18.33 ± 2.42              | 1.82                                     |
| Normal Female    | 16    | 10.09 ± 2.69              |                                           |

### Table 3. Average penis height on normal male and pseudopenis of imposex female.

| Jenis Betina     | Average penis or pseudopenis height ± SD (mm) | RPSI(%) |
|------------------|-----------------------------------------------|---------|
| Male             | 3.46 ± 1.5                                    |         |
| Imposex female   | 4.26 ± 1.3                                    | 187.96% |

Evidence to date suggests that imposex is generally caused by tributyltin (TBT) [8][9][10], a manufactured chemical massively used in self-polishing antifouling paints, which until recently was the most effective solution to prevent fouling on boat hulls [11]. Numerous investigations have linked imposex in Neogastropods with TBT contamination from antifouling paint in the marine environment [12][13][14][15].

As the evidence of 64.58% imposex among female *Turbo* sp it is suggested that TBT from antifouling paints on small ships seems the most likely source of imposex in the populations of Ujungpiring Waters, Jepara. The present results corroborate a previous study and confirm the high contamination detected in Demak waters [16]. This phenomenon is known to be induced by TBT at very low ambient concentrations, just a few ng/l are sufficient and imposex development seems dose-dependent [17]. Furthermore, high correlations between likely sources of TBT and imposex induction have been confirmed in several studies by chemical analyses of water, sediments and gastropod tissues. For instance, significant correlations between TBT concentration in seawater and imposex indices have been reported for *Nucella lapillus* [18][19], *Thais clavigera* [20], *Hexaplex trunculus* [21], *S. haemastoma* [22], and *Stramonita haemastoma* [23]. This sexual aberration is mainly attributable to TBT in seawater [22][24]. Imposex levels and organotin body burden can be assumed to be highest in populations nearest to major shipping activities and gradually lower with increasing distance from these sources [25][26].

### 4. Conclusion

In summary, imposex was recorded in *Turbo* sp. from three sites of the Ujungpiring waters of Jepara Regency. The evidence of imposex female *Turbo* sp. was 64.58%. Relative Penis Size Index (RPSI) of
Turbo sp. From Ujungpiring Waters was 187.86%. The smallest shell height of female imposex was 14.65 mm suggested the young age of gastropod affected by TBT.

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