Cah. Biol. Mar. (2006) 47 : 165-175

# Intensity of the imposex phenomenon - impact on growth and fecundity in *Hexaplex trunculus* (Mollusca: Gastropoda) collected in Bizerta lagoon and channel (Tunisia)

Najoua Trigui EL MENIF[1], Youssef LAHBIB[1], Marcel LE PENNEC[2], Roger FLOWER[3] and Moncef BOUMAIZA[1]

(1) *Université de Carthage, Faculté des Sciences de Bizerte, Département de Biologie, Laboratoire d'hydrobiologie, Tunisie, e-mail: elmunif2004@yahoo.fr*

(2) *Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, Technopôle Brest Iroise, F-29280 Plouzané, France*

(3) *University College London, ECRC, Geography, 26, Bedford Way, London, UK*

**Abstract:** One hundred individuals of the murex *Hexaplex trunculus* were sampled monthly at two stations in Bizerta lagoon (Tunisia) from January to July 2003. The station of Menzel Jemil was located in the east side of Bizerta lagoon and the one of the cement factory in the channel. Total length of shells varied from 40 to 60 mm. Temperature, salinity, dissolved oxygen and pH were simultaneously measured during the gastropod samplings. Results on the relative growth of the shell opening and on the dry flesh revealed that both bio-measures were higher in the lagoon than in the channel. However, weight growth of the shell was higher in the channel for individuals beyond 60 mm long. Comparing females between them and males between them showed that both dry flesh and dry shell growth was higher for individuals from the cement factory site beyond a certain size. Examination of the soft part of 100 to 150 specimens collected in January, March and June 2003 showed that the rate of imposex was 100% at the station of the cement factory but only 65.8% at the Menzel Jemil site. The intensity of development of the male genital tract in the females (imposex) seemed to have an impact on murex fecundity. Following a gamete emission (which proceeded in aquarium) during the period of spawning (February to June), the rate of specimens emitting the capsules as well as the average fecundity were more prevalent in the lagoon than in the channel. It is concluded that the exaggerated development of the male genital tract, primarily the vas deferens, in females at the cement factory station leads to a reduction of the gamete emission.

**Résumé :** *Intensité du phénomène d'imposex – impact sur la croissance et la fécondité d'*Heraplex trunculus *(Mollusca : Gastropoda) récolté dans la lagune de Bizerte et le canal de communication à la Méditerranée (Tunisie).* Des prélèvements mensuels de 100 individus du murex *Hexaplex trunculus*, de 40 à 60 mm de hauteur, ont été réalisés de janvier à juillet 2003 dans deux stations situées au nord de la Tunisie: celle de Menzel Jemil, sur le bord est de la lagune de Bizerte et celle de la cimenterie, située dans le canal de communication de la lagune avec la Méditerranée. Les paramètres physico-chimiques (température, salinité, oxygène et pH) ont été relevés mensuellement durant cette période d'étude. Le suivi de la croissance relative de l'ouverture de la coquille, de la chair et de la coquille sèche a révélé que chez les individus de la lagune, la croissance de l'ouverture et celle de la chair sèche est plus élevée que chez les individus du canal. En revanche, la croissance de la coquille est plus élevée chez les individus du canal pour les individus supérieurs à 60 mm. En compa-

Reçu le 15 juin 2005 ; accepté après révision le 11 mai 2006.
Received 15 June 2005; accepted in revised form 11 May 2006.

rant les femelles entre elles et les mâles entre eux, nous avons constaté que la croissance de la chair et de la coquille sèche est supérieure à la station de la cimenterie à partir d'une certaine taille de coquille. L'examen de la partie molle de 100 à 150 individus récoltés en janvier, mars et juin a montré que le taux d'imposex est de 100% à la station de la cimenterie contre 65,8% à celle de Menzel Jemil. L'intensité du développement du tractus génital mâle chez les femelles semble avoir un impact sur la fécondité du gastéropode. Suite à une émission gamétique qui s'est déroulée en aquarium pendant la période de ponte (de février-mars à juin), nous avons relevé que le taux d'individus émettant les capsules, ainsi que la fécondité moyenne, sont plus importants en lagune qu'en canal. Le développement exagéré du tractus génital mâle des femelles de la station de la cimenterie, notamment celui du canal déférent du côté de l'orifice de ponte, a un impact négatif sur l'émission gamétique.

*Keywords:* Mollusc; Gastropod; *Hexaplex trunculus*; Imposex**;** Growth; Fecondity

## Introduction

*Hexaplex trunculus* Linnaeus, 1758 (Neogastropod Mollusc) is abundant in the coastal area of Tunisia from 0 to 50 m depth. Its exploitation started in 1994 as a consequence of the problem of a phycotoxin which affected the local clam *Ruditapes decussatus* Linnaeus, 1758. Harvesting of this clam was then forbidden because of its toxicity. *H. trunculus* is however exposed, in certain sites of the littoral, to various contaminants from agriculture, industry, and urbanisation and also to molecules resulting from antifouling paintings (Lahbib et al., 2004).

The Channel of Bizerta is known for its intensive shipping traffic as several tankers cross it to the cement factory, the port of El Fouleth, in the lagoon, and also by the traffic of numerous steamers.

Anti-fouling paints applied on ship-hulls contaminate sea water by mean of compounds based on tin. Alzieu (2000) estimated that TBT concentrations from 1 ng to 500 µg.l$^{-1}$ caused disturbances in several marine species. According to this author, a concentration of 1 to 100 µg.l$^{-1}$ has an impact on the reproduction and the modification of the behaviour of fish.

Ferrua (1995) noted a disturbance in the cellular division of the sea urchin *Paracentrotus lividus* Lamarck, 1816 contaminated by tributyltin (TBT). In addition, Villa et al. (2003) observed a modification of the gametes structure of *Ascidia malaca* Traustedt, 1883 contaminated by stannic compounds.

Marine gastropods are the more sensitive species to the stannic compounds. There are a lot of reports on several species which exhibit secondary sexual characteristics and/or have reproductive abilities impacted by environmental endocrine disrupters. These anomalies appeared in the occurrence of the toxic substances coming from the progressive leaching of anti-fouling paints covering the hulls of boats (Bryan & Gibbs, 1991). More than 150 species of Neogastropods exhibited imposex (Schulte-Oehlmann et al., 2000) but, according to Spooner et al. (1991), this phenomenon expressed a male genital tract in the females when hormonal balance was affected. This imbalance could be due to the inhibition of the P450 cytochrome by TBT (Bettin et al., 1996). According to these authors this pollutant was able to fix itself against the aromatase of the cytochrom, in place of the male steroid hormone. The present study contributes to examine the environmental conditions affecting imposex development in *Hexaplex trunculus* during the spawning period.

## Material and methods

Temperature, salinity, dissolved oxygen and pH were measured monthly in two stations where 100 individuals of *Hexaplex trunculus* were sampled from January to July 2003. The equipment used was a WTW.198 multilab type. The size of the gastropods ranged from 20 to 60 mm. Sampling stations were different according to environmental conditions and sea traffic activities. The cement factory (or channel) station (Fig. 1), was located in Bizerta channel (37°16'-37°15'N; 9°53'-9°52'E) and that of Menzel Jemil (also named lagoon) in the south-eastern sector of Bizerta lagoon (41°25'-41°41'N; 8°27'-8°46'E).

A total of 700 specimens of *H. trunculus* per station were used for the relative growth study. The imposex intensity was evaluated by using 100 to 150 individuals ranged from 40 to 60 mm long. The soft part of the animals was withdrawn of the shell. Occurrence of a male genital tract in some females forced us to check the presence of the capsule gland. The gonad encrusted in the digestive gland was observed under a microscope and males and females were separated. The penis of the males and of the abnormal females, including the whip, was measured with a micrometric binocular. The rate of imposex, RPLI (Relative Penis Length Index) and VDSI (Vas Deferens Sequence Index) were calculated as follow:

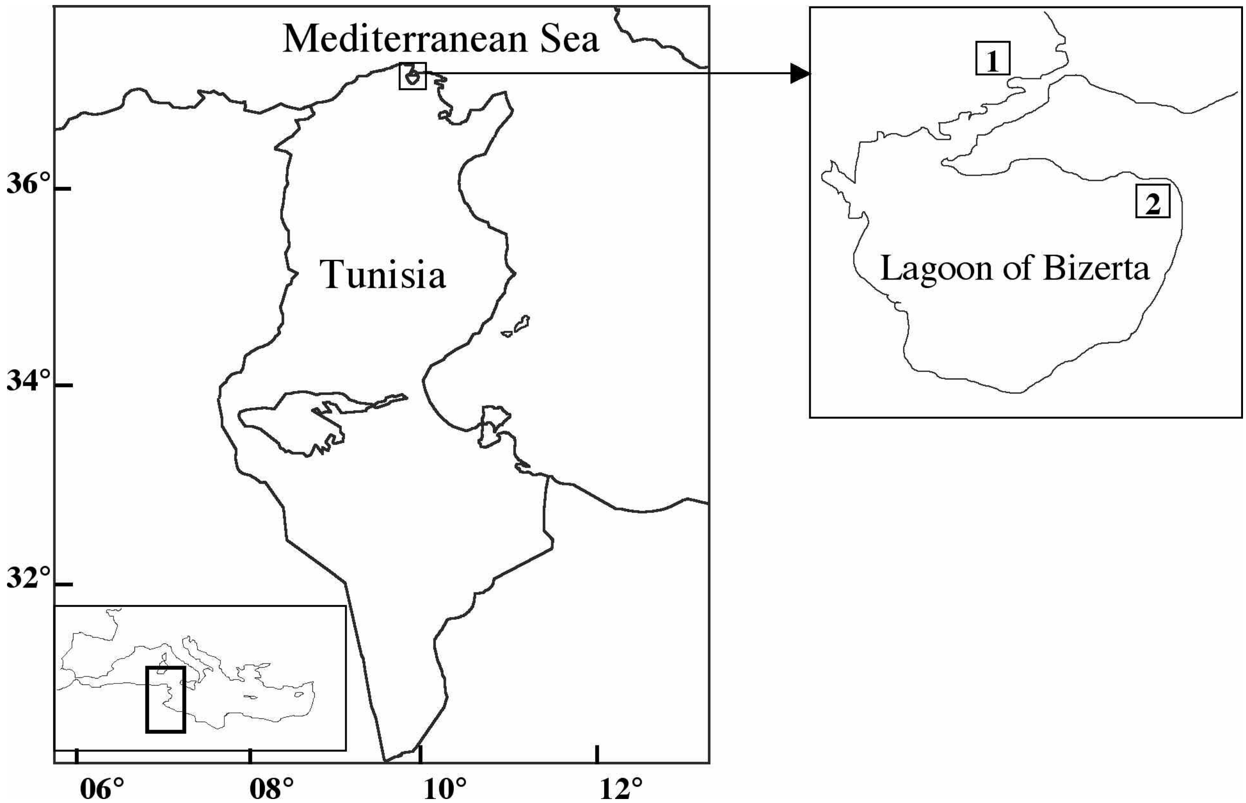


**Figure 1.** Map of Tunisia, sampling sites: *Hexaplex trunculus.* 1: Site of cement factory (Bizerta channel), 2: Site of Menzel Jemil (lagoon of Bizerta)

**Figure 1.** Carte de la Tunisie montrant les stations de prélèvement d'*Hexaplex trunculus*. 1: Station de la cimenterie (canal de Bizerte), 2 : Station de Menzel jemil (lagune de Bizerte).

$$F = \frac{\text{Number of abnormal females}}{\text{Total number of females}} \times 100 \qquad (1)$$

(Michin et al., 1996)

$$RPLI = \frac{\text{Average length of the penis of females}}{\text{Average of the length of the penis of males}} \times 100 \qquad (2)$$

(Axiak et al., 1995)

$$VDSI = \frac{\text{Sum of stages observed}}{\text{Total number of females}} \qquad (3)$$

VDSI was calculated under binocular microscope from the detailed observations of the development level of the genital tract (vas deferens and penis). Results enabled us to applied the VDS scale (Vas Deferens Sequence) proposed by Gibbs et al. (1987) on *Nucella lapillus* Linnaeus, 1758 and completed thereafter by Stroben et al. (1992) on *Hinia reticulata* Linnaeus, 1758 and by Axiak et al. (1995) and Terlizzi (1999) on *Hexaplex trunculus*.

As for the relative growth, results were compared between the populations of both stations. Initially, males and females were considered together, then the relative growth comparison was studied between males and females belonging to each station. A third comparison of the relative growth was carried out in order to compare females between them and males between them. The relative growth of the shell opening, the dry shell and the dry flesh were compared to the total height (H) of the gastropods. Morphometric measurements of the parameters: shell opening (O), H and shell and dry flesh weight (Wcq-s and Wch–s respectively) were measured using a slide caliper (10 mm graduation) and a precision balance. The dry weight was obtained by drying the flesh in an oven maintained at 60°C until the constant weight was obtained.

The growth speed was obtained by applying the following equation:

$$Y = a\,x^{b} \qquad (4)$$

(a: condition index; b: allometric index; y: risky dependent variable presenting dry flesh weight; x: independent variable presenting the reference parameter).

When linear mensurations are used, the valour of b is assimilated to the valour 1. As we compare the relative increasing of weight with height, the slope value is 3. b is inferior, or superior to 1 (linear parameters) or to 3 (weight parameters). We have determined the nature of allometry by comparing the value of the slope to the theoretical value using Student test with the threshold of probability of 5%. In order to compare the growth of *Hexaplex trunculus* in the two stations and between sexes, we used the tests of slope (tpe) and position (tpo) (Mayrat, 1959). In January 2004, we sampled, in each station, 100 to 150 individuals with a size ranging from 40 to 60 mm with the aim of determining and comparing fecundity. The samples were placed in 4 aquariums of 50x20 cm containing each one 50 individuals. The sea water was renewed twice a week and the gastropods were fed with clams (*Ruditapes decussatus).* Sea water and food were collected near the two sampling sites and water oxygenation and filtration were continued. Fecundity was given following the counting of the number of capsules per spawning and the number of eggs per capsule. At least, the capsules, which were attached each others by their base were isolated for counting. The draining of each capsule in Dolfus cuve enabled us to count eggs.

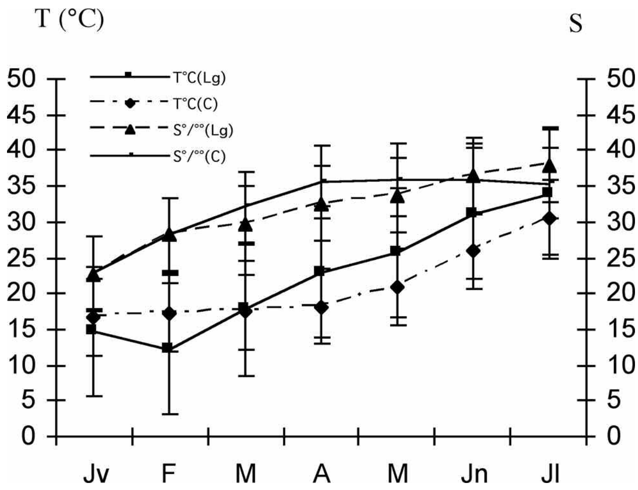


**Figure 2.** Monthly variations of the temperature and salinity in the stations of the cement factory (C) and Menzel-Jemil (Lg).

**Figure 2.** Variations mensuelles de la température et de la salinité dans les stations de la cimenterie (C) et de Menzel jemil (Lg).

## Results

*Environmental parameters evolution (Table 1)*

Temperature (Fig. 2). The average temperature value recorded in Menzel-Jemil during the sampling period was 24.1°C. The minimum, 12.2°C, was recorded in February and the maximum, 33.9°C, in July. In the cement factory site, the average temperature was 21°C. The minimal, 16.7°C, was obtained in January and the maximum, 30.6°C, recorded in July. The thermal variation in Menzel-Jemil, 19.2°C, was higher than in the channel station, 13.9°C.

Salinity (Fig. 2). The lowest values, 22.7, were recorded in January at Menzel-Jemil and 22.9 at the other station. This low value was explained by an important rainfall during this period (135.7 mm). The maximum value of salinity, 37.9 was recorded in June for Menzel-Jemil and in July for the cement factory site, 36. The margin of the salinity which was more important in Menzel-Jemil, 15.2, than in the other station, 13.1, was explained by the influence of the climatic variations on the shallow waters of the coast.

Dissolved oxygen (Fig.3). The dissolved oxygen content in water varied from 5.1 to 9.8 $mg.l^{-1}$ in the station of Menzel-Jemil with an average of 8.35 $mg.l^{-1}$, and from 6.1 to 9.3 $mg.l^{-1}$ in the cement factory site (average value of 7.14 $mg.l^{-1}$).

pH (Fig. 3). The pH was relatively stable in the two stations. Values were ranged from 8.05 to 9.82 in Menzel-Jemil (average of 8.65) and from 7.64 to 8.36 in the second site (average of 8.07). Indeed, the weak variation of the pH depended on several factors such as the bacteriological activity, photosynthesis, temperature and salinity (Ghribi, 2003).

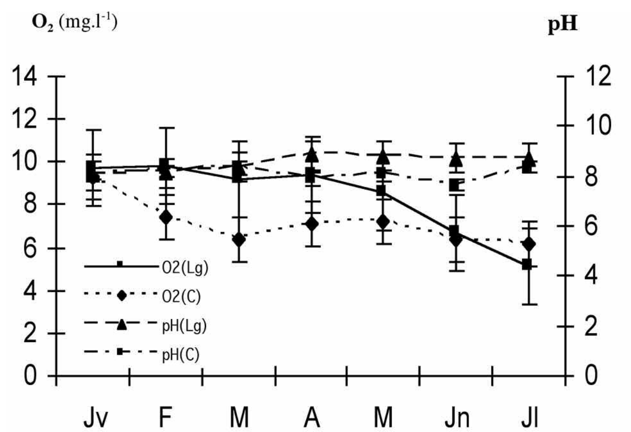


**Figure 3.** Monthly variations of dissolved oxygen and the pH in the stations of the Cement factory (C ) and Menzel-Jemil (Lg).

**Figure 3.** Variations mensuelles de l'oxygène dissous et du pH dans les stations de la cimenterie (C ) et de Menzel-Jemil (Lg).

*Rate and intensity of imposex variation*

The rate and the imposex intensity observed changed from one station to another (Table 2). Individuals in the cement factory site were more affected than those of Menzel-Jemil. The rate of imposex recorded was 100% against 65.8%. The average length of the penis of the females showed a

**Table 1.** Monthly values of the physicochemical parameters (Temperature, Salinity, dissolved Oxygen and pH) in the two stations during the period from January to July 2003. Moy: mean values, s.d.: standard deviation.

**Tableau 1.** Valeurs mensuelles des paramètres physico-chimiques (Température, Salinité, Oxygène dissous et pH) mesurées de janvier à juillet dans les deux stations. Moy : valeurs moyennes, s.d. : écart-type.

| | January | February | Mars | April | May | June | July | **Moy** | **s.d.** |
|---|---|---|---|---|---|---|---|---|---|
| **T (°C) Lagoon** | 14.7 | 12.2 | 17.7 | 33.2 | 25.7 | 31.2 | 33.9 | 24.1 | 9.1 |
| **T (°C) Channel** | 16.7 | 17.2 | 17.4 | 18.2 | 20.9 | 26 | 30.6 | 21 | 5.3 |
| **S Lagoon** | 22.7 | 28.2 | 29.8 | 32.6 | 33.7 | 36.5 | 37.9 | 31.6 | 3.7 |
| **S Channel** | 22.9 | 28.3 | 32.1 | 35.6 | 35.9 | 36 | 35.4 | 32.3 | 5 |
| **$O_2$ ($mg.l^{-1}$) Lagoon** | 9.7 | 9.8 | 9.2 | 9.4 | 8.6 | 6.7 | 5.1 | 8.4 | 1.8 |
| **$O_2$ ($mg.l^{-1}$) Channel** | 9.3 | 7.4 | 6.4 | 7.1 | 7.2 | 6.4 | 6.2 | 7.1 | 1.1 |
| **pH Lagoon** | 8 | 8.1 | 8.3 | 8.8 | 8.8 | 8.7 | 8.7 | 8. 5 | 0.3 |
| **pH Channel** | 8.1 | 8.2 | 8.3 | 7.9 | 8 | 7.6 | 8.4 | 8.1 | 0.3 |

**Table 2.** Variation of the intensity of imposex in two stations: Menzel-Jemil (Lagoon of Bizerta) and of the Cement factory (Channel of Bizerta). F: frequency, H: height of gastropoda (mm), Lfp: length of female penis (mm), Lmp: length of male penis (mm), s.d.: standard deviation, RPLI: Relative Penis Length Index, VDSI: Vas Deferens Sequence Index.

**Tableau 2.** Variation de l'intensité du phénomène d'imposex dans les deux stations : Menzel jemil (lagune de Bizerte) et la cimenterie (canal de Bizerte). F : fréquence, H : la hauteur du néogastéropode, Lfp : longueur moyenne du pénis femelle, Lmp : longueur moyenne du penis mâle (mm), s.d. : écart type, RPLI : Relative Penis Length Index, VDSI : Vas Deferens Sequence Index.

| | | F% | H (f) | s.d. | Lfp | s.d. | H (m) | s.d. | Lmp | s.d. | RPLI | VDSI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jv | 66.5 | 51.9 | 4.1 | 0.68 | 0.7 | 46.4 | 4 | 19 | 3.8 | 3.6 | 0,31 |
| Lagoon of Bizerta | Ms | 65 | 45.5 | 2.4 | 0.29 | 0.2 | 52.1 | 2.5 | 15.21 | 2.6 | 1.8 | 0,35 |
| (St. Menzel-Jemil) | Jn | 66.6 | 42.6 | 2.6 | 0.28 | 0.2 | 47.4 | 4.6 | 13.56 | 2.9 | 2,1 | 0,3 |
| | **Moy** | **65.8** | **46.2** | **4.3** | **0.41** | **0.4** | **49** | **4.2** | **14.75** | **3.5** | **2.5** | **0,34** |
| | Jv | 100 | 45.3 | 2.4 | 11.02 | 4.5 | 41.4 | 1,1 | 17.72 | 3.2 | 62.6 | 4,09 |
| Channel of Bizerta | Ms | 100 | 44.7 | 2.4 | 10.21 | 3.8 | 43.3 | 3.7 | 16.23 | 2.8 | 62.9 | 4,13 |
| (St.Cement factory) | Jn | 100 | 44.1 | 2.6 | 8.23 | 3,6 | 44.2 | 2.4 | 12.05 | 5.4 | 68.2 | 4,24 |
| | **Moy** | **100** | **44.7** | **2.5** | **9.8** | **4.8** | **43.1** | **2.7** | **16.58** | **4.7** | **64.6** | **4,19** |

difference in favour of the specimens from the channel station. The average value of 9.82 mm was recorded in the females of the cement factory site and 0.41 mm in the females from Menzel-Jemil. The important average size of the male and female penis was recorded in January. Between stations, the average size was higher in the channel than at Menzel-Jemil (Table 2).

Index RPLI values were higher in the cement factory station than at Menzel-Jemil, whereas the values were ranged from 1.8 in March to 3.57 in January, with an average of 2.47. It was pointed out that the higher value of RPLI recorded in January at Menzel-Jemil was primarily due to the female size of the penis, which was larger (Table 2).

The weak values of VDSI recorded at Menzel-Jemil indicated that the environmental area was slightly contaminated compared to the cement factory site where the values of VDSI were ranged from 4 to 5. Posteriorly, we get a rate of sterility of 8% due to a crack in the capsule gland.

*Relative growth variation*

<u>Growth of opening according to height</u>.The comparison of the slopes of the straight regression lines showed that the shell opening grew more quickly than the height in the individuals of both stations. The growth between stations was slightly different and was in favour of the individuals of Menzel-Jemil (Table 3A, Table 5). Between males and females of the same station (Table 3B, Table 5), the females have an important shell opening at Menzel-Jemil whereas in the channel site, the phenomenon was reversed in favour of the males starting from a height of 40 mm. With females compared between them and males compared between them (Table 4A-B, Table 5), the growths were similar and the differences were significant only on the level of the position. For any height of the gastropod, the opening was larger in the females of Menzel-Jemil (Table 5).

<u>Growth of dry flesh weight according to height</u>. The growth of the weight of the dry flesh was significantly higher than

**Table 3.** Values of the parameters of the equations binding the opening (O), the weight of dry flesh (Wch-s) and the weight of dry shell (Wcq-s) with the height (H) in the two stations. (A): Without holding account of the sex; (B): by holding account of the sex. a: coefficient of allometry, b: index of the origin, r: coefficient of correlation, t: test of Student, tpe: test of slope, tpo: test of position, (+, -): statistical significance with the threshold of 5%.

**Tableau 3.** Valeurs des paramètres des équations liant l'ouverture (O), le poids de la chair sèche (Wch-s) et le poids de la coquille sèche (Wcq-s) à la hauteur (H) dans les deux stations. (A) : sexe confondu, (B) : en fonction du sexe, a : coefficientd'allométrie, b : indice d'origine, r : coefficient de corrélation, t: test de Student, tpe: test de pente, tpo: test de position, (+, -): significatif ou non significatif au seuil de probabilité de 5%.

| | | A | | B | | | |
|---|---|---|---|---|---|---|---|
| Station | | Menzel-jemil | Cement factory | Menzel- jemil | | Cement factory | |
| | | | | female | male | female | male |
| Relation O /H | $y = bX^a$ | $O = 0.154H^{1.126}$ | $O = 0.154H^{1.104}$ | $O = 0.155H^{1.125}$ | $O = 0.153H^{1.127}$ | $O = 0.171H^{1.075}$ | $O = 0.144H^{1.122}$ |
| | r | 0.9386 | 0.9567 | 0.945 | 0.925 | 0.96 | 0.947 |
| | t | 7.46 (+) | 7.51 (+) | 5.840 (+) | 4.498 (+) | 6.557 (+) | 3.507 (+) |
| | tpe | 1.004 (-) | | 0.059 (-) | | 1.652 (-) | |
| | tpo | 24.44 (+) | | 0.746 (-) | | 0.26 (-) | |
| Relation Wch-s/H | $y = bX^a$ | $Wchs = 7.10^{-6}H^{3.179}$ | $Wchs = 2.10^{-6}H^{3.448}$ | $Wchs = 5.10^{-6}H^{3.238}$ | $Wchs = 10^{-6}H^{3.086}$ | $Wchs = 2.10^{-6}H^{3.486}$ | $Wchs = 3.10^{-6}H^{3.384}$ |
| | r | 0.876 | 0.916 | 0.911 | 0.832 | 0.918 | 0.911 |
| | t | 108.39 (+) | 312.23 (+) | 136.19 (+) | 27.04 (+) | 235.74 (+) | 195.24 (+) |
| | tpe | 122.75 (+) | | 1.021 (-) | | 35.7 (+) | |
| | tpo | - | | 38.59 (+) | | | |
| Relation Wcq-s/H | $y = bX^a$ | $Wcoqs = 2.10^{-4}H^{2.671}$ | $Wcoqs = 10^{-4}H^{2.841}$ | $Wcoqs = 2.10^{-4}H^{2.665}$ | $Wcoqs = 2.10^{-4}H^{2.683}$ | $Wcoqs = 9.10^{-5}H^{2.875}$ | $Wcoqs = 10^{-4}H^{2.882}$ |
| | r | 0.978 | 0.934 | 0.958 | 0.938 | 0.951 | 0.925 |
| | t | 142.7 (+) | 30.9 (+) | 55.772 (+) | 54.618 (+) | 79.57 (+) | 14.40 (+) |
| | tpe | 30.22 (+) | | 0.06 (-) | | 0.684 (-) | |
| | tpo | - | | 41.96 (+) | | 4.55 (+) | |

**Table 4.** Values of the parameters of the equations binding the opening (O), the weight of the dry flesh (Wch-s) and the weight of dry shell (Wcq-s) with the height (H). (A): between females of the two stations, (B): between males of the two stations, a: coefficient of allometry, b: index of the origin, r: coefficient of correlation, t: test of Student, tpe: test of slope, tpo: test of position, (+, -): statistical significance with the threshold of 5%.

**Tableau 4.** Valeurs des paramètres des équations liant l'ouverture (O), le poids de la chair sèche (Wch-s) et le poids de la coquille sèche (Wcq-s) à la hauteur (H) dans les deux stations. (A) : entre femelles des deux stations, (B) : entre mâles des deux stations, a : coefficientd'allométrie, b : indice d'origine, r : coefficient de corrélation, t : test de Student, tpe : test de pente, tpo : test de position, (+, -): significatif ou non significatif au seuil de probabilité de 5%.

| **A** | | female (Menzel- Jemil) | female (Cement factory.) | **B** | | male (Menzel-jemil) | male (Cement factory) |
|---|---|---|---|---|---|---|---|
| Relation | $y = bX^a$ | $O = 0.155H^{1.125}$ | $O = 0.171H^{1.075}$ | Relation | $y = bX^a$ | $O = 0.153H^{1.127}$ | $O = 0.144H^{1.122}$ |
| O/H | r | 0.945 | 0.96 | O /H | r | 0.925 | 0.947 |
| | t | 5.840 (+) | 6.557 (+) | | t | 4.498 (+) | 3.507 (+) |
| | tpe | 0.528(-) | | tpe | | 0.141(-) | |
| | tpo | 17.62(+) | | tpo | | 16.32 (+) | |
| Relation | $y = bX^a$ | $Wchs = 5.10^{-6} H^{3.238}$ | $Wchs = 2.10^{-6} H^{3.486}$ | Relation | $y = bX^a$ | $Wchs = 10^{-5} H^{3.086}$ | $Wchs = 3.10^{-6}H^{3.384}$ |
| Wch-s/H | r | 0.911 | 0.918 | Wch-s/H | r | 0.832 | 0.911 |
| | t | 136.19 (+) | 235.74 (+) | | t | 27.04 (+) | 195.24 (+) |
| | tpe | 91.71(+) | | tpe | | 79.17(+) | |
| | tpo | - | | tpo | | - | |
| Relation | $y = bX^a$ | $Wcoqs = 2.10^{-4}H^{2.665}$ | $Wcoqs = 9.10^{-5}H^{2.875}$ | Relation | $y = bX^a$ | $Wcoqs = 2.10^{-4}H^{2.683}$ | $Wcoqs = 10^{-4}H^{2.882}$ |
| Wcq-s/H | r | 0.958 | 0.951 | Wcq-s/H | r | 0.938 | 0.925 |
| | t | 55.77 (+) | 79.57 (+) | | t | 54.62 (+) | 14.40 (+) |
| | tpe | 28.17(+) | | tpe | | 19.896 (+) | |
| | tpo | - | | tpo | | - | |

**Table 5.** Values of the opening, of the weight of the dry flesh and the weight of the dry shell starting from certain values the height (H mm). O: opening, Wd-f: weight of the dry flesh, Wd-s: weight of the shell dry.

**Tableau 5.** Valeurs de l'ouverture, du poids de la chair sèche et de la coquille calculés à partir des valeurs standard de la hauteur (H mm). O : ouverture, Wd-f : poids de la chair sèche, Wd-s : poids de la coquille sèche.

| Parameter | | 10 | 20 | 30 | 50 | 70 |
|---|---|---|---|---|---|---|
| aperture | **pop.Mz-J** | **2.06** | **4.49** | **7.09** | **12.60** | **18.41** |
| | fem.Mz-J | 2.07 | 4.5 | 7.11 | 12.64 | 18.45 |
| | mal.Mz-J | 2.04 | 4.47 | 7.07 | 12.57 | 18.37 |
| | **pop.Cem.f** | **1.95** | **4.20** | **6.58** | **11.56** | **16.76** |
| | fem.Cem.f | 2.03 | 4.28 | 6.62 | 11.46 | 16.46 |
| | mal.Cem.f | 1.9 | 4.15 | 6.54 | 11.6 | 16.92 |
| Wd-f | **pop.Mz-J** | **0.011** | **0.096** | **0.35** | **1.76** | **5.13** |
| | fem.Mz-J | 0.0086 | 0.082 | 0.303 | 1.58 | 4.71 |
| | mal.Mz-J | 0.012 | 0.103 | 0.36 | 1.75 | 4.9 |
| | **pop.Cem.f** | **0.0056** | **0.061** | **0.247** | **1.44** | **4.60** |
| | fem.Cem.f | 0.006 | 0.068 | 0.282 | 1.67 | 5.41 |
| | mal.Cem.f | 0.0073 | 0.076 | 0.29 | 1.68 | 5.26 |
| Wd-s | **pop.Mz-J** | **0.094** | **0.597** | **1.76** | **6.90** | **16.95** |
| | fem.Mz-J | 0.092 | 0.586 | 1.72 | 6.74 | 16.52 |
| | mal.Mz-J | 0.096 | 0.62 | 1.84 | 7.23 | 17.84 |
| | **pop.Cem.f** | **0.069** | **0.496** | **1.57** | **6.71** | **17.45** |
| | fem.Cem.f | 0.067 | 0.495 | 1.58 | 6.9 | 18.15 |
| | mal.Cem.f | 0.076 | 0.56 | 1.81 | 7.88 | 20.77 |

the growth height (Table 3A-B). The value of tpe, 122.75, between specimens of Menzel-Jemil and those of the cement factory station indicated that the growth was different in the two stations, and was in the benefit of the lagoon population (Table 5).

For the males and females from the same station (Table 3A-B), the values of the tests showed that growth was the same in Menzel-Jemil and that the difference, in favour of the males, only existed on the level of the position. On contrary, in the cement factory station, growth was significantly different, and was in favour of the females starting from 50 mm high (Table 5). By comparing the females of the two stations as well as the males between them (Table 4A-B), we noticed that growth was different, and was in favour of the females of Menzel-Jemil beyond a size of 40 mm (Table 5). For the males from the canal site, dry flesh weight became higher beyond 55 mm size.

<u>Growth of dry shell weight according to height</u>. Growth of the shell of individuals sampled at Menzel-Jemil and cement factory stations was significantly lower than that of height, as attested by the values of the Student test (Table 3A). The significant difference of the growth was in favour of individuals from the channel (Table 5). Between males and females of the same station, a difference was recorded in the position, in favour of the males (Table 3B, Table 5). Females compared between them as well as the males between them (Table 4A-B) showed a difference in growth, in favour of females of the channel, higher then 45 mm and for males higher from 29-30 mm (Table 5).

*Spawning and fecundity*

Placed in aquariums individuals from Menzel-Jemil began to spawn in February, whereas those from the channel started emitting capsules in the beginning of March. Spawning by one gastropod seemed to influence the others which moved toward it and then started emitting capsules against the initial emission so that the spawned quantity keeps on increasing (Fig. 4).

It was pointed out that spawning continued until July. The density of individuals emitting the capsules was greater in the lagoon, 30%, with 50% mortality, whereas in the channel the rate was 12% with a 8% mortality. This observation was made after a considerable spawned quantity was recorded in the two aquariums containing the specimens from Menzel-Jemil.

The volume spawned and recovered from the aquarium differed significantly especially from the lagoon individuals. A capsule counted per emission showed that the average value was much higher in lagoon females (Table 6). The high values of the standard deviation showed great individual variations.

As for the number of capsules per unit of volume, the values recorded were slightly in favour of *Hexaplex trunculus* from the canal (Table 6). But the number of eggs per capsule was significantly higher in the lagoon rather than in the channel, leading thus to the assumption that the average size of the capsules emitted in the second site was smaller.

## Discussion

Study of the relative growth of the two populations of murex gastropods collected from the Bizerta lagoon and the channel made it possible to highlight the influences of several environmental factors on the biology of this mollusc. First, growth of the shell's opening was greater in the lagoon individuals than in the channel, assuming thus that the soft part biomass of the neogastropod from this last site was less than that from Menzel-Jemil. This was shown after a study of the relative growth of the dry body weight in relationship to the shell height. The soft part of Menzel-Jemil gastropods was more developed than that from the channel, reaching a size of 40 mm in females and 50 mm in males. The results obtained for the growth of the shell

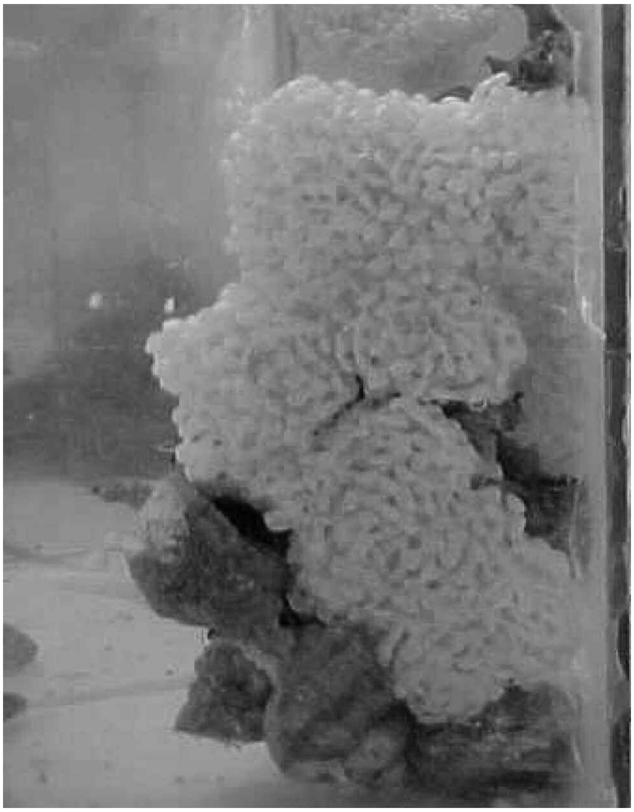

**Figure 4.** Spawning of *Hexaplex trunculus.*
**Figure 4.** Pontes de *Hexaplex trunculus.*

**Table 6.** Variation of fecundity from *Hexaplex trunculus* collected in Menzel-Jemil and the cement factory. Nm: average numbers capsules by lying, s.d.: standard deviation, Nmv: average numbers capsules per unit of volume, Nec: average numbers of embryon by capsule.

**Tableau 6.** Variation de la fécondité de *Hexaplex trunculus* collecté dans les stations de Menzel-Jemilet de la cimenterie. Nm : nombre moyen de capsules par ponte, s.d. : écart-type, Nmv : nombre moyen de capsules par unité de volume, Nec : nombre moyen d'embryon par capsule.

| | Nm | s.d. | Nmv ($ml^{-1}$) | Nec | s.d. |
|---|---|---|---|---|---|
| Menzel-Jemil | 960 | 402 | 57 | 459 | 26 |
| Cement factory | 255 | 108 | 60 | 281 | 61 |

showed that it became heavier once the neogastropod had reached a certain size, 29-30 mm in males and 45 mm in females.

Growth differences could be due to environmental conditions variations at the two stations even if the values recorded for salinity, dissolved oxygen and pH were very similar. As for temperature, a difference of 3.1°C was recorded between the two stations. Navarro et al. (2002) observed that the temperature and diet constituted two determinant factors in the somatic and/or gonadic growth of the gastropod *Chorus giganteus* Lesson, 1830. The same authors demonstrated that the gonadic development was at its maximum when the animals feed on the bivalve *Tagelus dombeii* Lamarck, 1818 rather than on *Mytilus chilensis* Hupe, 1854. As for the two Tunisian stations, the food was highly diversified. In Menzel-Jemil as, apart from decomposed dead bodies, the prey of *Hexaplex trunculus* basically comprises *Ruditapes decussatus, Cerithium vulgatum* Bruguiere, 1792 and crabs: *Carcinus maenas* Linnaeus, 1758. In the channel, the preys were mainly represented by *Cerithium vulgatum*.

Furthermore, a thickening of the shell observed in the channel population was probably due to the presence of TBT in the environment. This pesticide has been widely used since the 1960s in anti-fouling paints covering the hulls of ships and its effects on the flora and fauna very soon made themselves felt because of the toxicity of this organostannic compound (Rosenberg et al., 1980). Various studies on *Nucellus lapillus* in British waters showed that TBT, in tiny doses of 1ng/l sea water, could affect the gastropod females by masculinizing them (Bryan et al., 1987).

Furthermore, Bryan & Gibbs (1991) and Evans et al. (1995) showed that organostannic compounds could produce adverse effects in several species of marine organisms such as the thickening of the shell. According to Bryan & Gibbs (1991), this shell anomaly observed in oysters collected along the pacific coast could lead to the death of these bivalves. In the abnormal *H. trunculus*, Terlizzi et al. (1999) noticed that at an advanced stage, increasing in size and width shell is greater compared with normal populations or those only partially affected by the imposex phenomenon. It is thus highly probable that these organostannic compounds, after a long period of exposure, have an effect on the thickness of the shell of these *Muricidae*, as the dry shell weight was greater in the canal (100% imposex) once the gastropod had reached a certain size. With *Buccinum undatum* Linnaeus, 1758 it was demonstrated that TBT causes various shell malformations resulting in abrasions and holes in the shell and a loss of the operculum leading to the animal's death (Mensink et al., 2000). Other studies have shown that this tin-based biocidal agent disrupted the calcification mechanisms in the oysters *Ostrea edulis* Linnaeus, 1758 and *Crassostrea gigas* Linnaeus, 1758, inducing the formation of additional shells separated by chambers filled with a gelatinous substance (Axiak, et al. 1995; Camillieri, 1995). According to Axiak et al. (2000), these shell anomalies were due to diminished somatic growth because of considerable atrophy of the digestive cells due to the effects of TBT.

Furthermore, the body weight was greater in females from the cement factory station when a size of 40 mm was attained. This could be explained by the fact that the development of a male genital tract in *Hexaplex* females with a VDSI above or equal to 4, provoked a reduction in fecundity and in the size of the capsules or led to non-emission because of a partial or total closing of the spawning orifice. This situation means that individuals could not expel their capsules normally and the soft part becomes more developed in affected individuals compared with normal ones, which expelled all their capsules. Son et al. (2000) observed that shell growth of imposexed *Nucella lapillus* populations was much more accentuated than for normal females. According to the same author, this difference was due to the energy meant for reproduction, being instead mobilized for the growth of the shell as TBT affects gonadic activity. As for *H. trunculus*, it is thought that the presence of a highly developed male genital tract in canal females reduced or prevented gamete emission. This can be confirmed by the presence of highly developed gonads for abnormal females of the channel collected in June and July.

Concerning the growth of the dry body weight, our result showed it was in favour of males from the canal site, starting from 55 mm. This could be probably due to the partial or total obstruction of the female spawning orifice, thus, preventing mating. In this case, the male could not spawn. It was also possible that the behaviour of the males changed in the presence of abnormal females.

We must continue this study by measuring the concentrations of TBT, DBT and MBT in sea water and in the body tissue of this species. In parallel it will be interesting to demonstrate the effects of stannic compounds on gonadic development and on the ultra-structural quality of the eggs of *H. trunculus*. This will be an opportunity to confirm other studies (Ferrua, 1995) where is was shown that in some ascidia TBT acts on the structure of gametes and in some sea urchins it acts on cell division of sea urchins (*Paracentrotus lividus*) eggs.

## References

**Alzieu C. 2000.** Impact of tributyltin on marine Invertebrate. *Ecotoxicology*, **9**: 71-76.

**Axiak V., Vella A.J., Micallef D, Chircop P. & Mintoff B. 1995.** Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Marine Biology*, **121:** 685- 691.

**Axiak V., Vella A.J., Agius D., Bonnici P., Cassar G., Cassone R., Chircop P., Micallef D., Mintoff B. & Sammut M. 2000.** Evaluation of environmental levels and biological impact of TBT in Malta (central Mediterranean). *The Science of the Total Environment*, **258:** 89-97.

**Bettin C., Oehlmann J. & Stroben E. 1996.** TBT induced imposex in marine neogastropods is mediated by increasing androgen level. *Helgoländer Meeresuntersuchungen*, **50**: 299-317.

**Bryan G.W., Gibbs P.E., Hammerstone L.G. & Burt G.R. 1987.** The effects of tributyltin accumulation on adult dogwelks, *Nucella lapillus*, long term field and laboratory experiments. *Journal of the Marine Biology Association of the United Kingdom*, **67:** 524-544.

**Bryan G.W. & Gibbs P.E. 1991.** Impact of low concentrations of tributyltin TBT on marine organisms: A review. In: *Ecotoxicology, Concepts and Applications* (M.C. Newman & A.W. Mcintosh, eds), pp. 323-361.

**Camilieri S. 1995.** A preliminary on the integration of fish and shellfish farming. *B. Sc. Dissertation. Department of Biology, University of Malta,* 230 p.

**Evans S.M., Leksoma T. & McKinnel P.D. 1995.** Tributyltin in scottish sea lochs, as indicated by degree of imposex in the dog-whelk, *Nucella lapillus* (L.). *Marine Pollution Bulletin,* **18**: 400-404.

**Ferrua C. 1995.** Effet du tributhylétain sur les mécanismes contrôlant la division cellulaire de l'œuf d'oursin *Paracentrotus lividus. Diplôme des études approfondies, Sciences de l'Environnement Marin*, 37 p.

**Ghribi S. 2003.** Etude de l'évolution spatio-temporelle du mesozooplancton dans le canal de Bizerte. *Diplôme des Etudes approfondies*, 110 p.

**Gibbs P.E., Bryan G.W., Pascoe P.L. & Burt G.R. 1987.** The use of the dog-whelk, Nucella lapillus, as an indicator of tributyltin (TBT) contamination. *Journal of the marine biological Association of the United Kingdom,* **67**: 507-523.

**Lahbib Y., Trigui El Menif N., Le Pennec M. & Boumaiza M. 2004.** Le phénomène d'imposex observé pour la première fois en Tunisie chez un mollusque gastéropode, *Murex trunculus. Rapport de la Commission internationale de la Mer Méditerranée*, **37**: 218.

**Mayrat A. 1959.** Nouvelle méthode pour l'étude comparée d'une croissance relative dans deux échantillons. Application à la carapace de *Panaeus kerathurus* (Forskal). *Bulletin de l'institut français de l'Afrique noire*, A, **21:** 21-59.

**Mensink B.P., Fischer C.V., Cadée G.C., Fonds M., Ten Hallers-Tjabbes C.C. & Boon J.P. 2000.** Shell damage and mortality in the common whelk *Buccinum undatum* caused by beam trawl fishery. *Journal of Sea Research,* **43:** 53-64.

**Minchin D., Stroben E., Oehlmann J., Bauer B., Duggan C. & Keatinge M. 1996.** Biological indicators used to map organotin contamination in Cork Harbour, Ireland. *Marine Pollution Bulletin*, **32:**188-195.

**Navarro J.M., Leiva G.E., Gallardo C.S & Valera C. 2002.** Influence of diet and temperature on physilogical energetics of *Chorus giganteus* (Gastropoda:Muricidae) during reproductive conditioning. *New Zealand Journal of Marine and Freshwater Research*, **36:** 321.

**Rosenberg D.W., Drummond G.S., Cornish H.C. & Kappas A. 1980.** Prolonged induction of hepatic haem oxygenase and decreases in cytochrome P-450 content by organotin compounds. *Biochemistry Journal,* **190:** 465-468.

**Schulte-Oehlmann U., Tillmann M., Markert B., Oehlmann J., Watermann B. & Scherf S. 2000.** Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the Laboratory. Part II: triphenyltin as a xeno-androgen.

*Ecotoxicology,* **9**: 399-412.

**Son M.H. & Hughe R.N. 2000.** Relationship Between Imposex and Morphological Variation of the Shell in *Nucella lapillus* (Gastropoda:Thaididae). *Estuarine, Coastal and Shelf Science*, **50:** 599-606.

**Spooner N., Gibbs P.E., Bryan G.W. & Goad L.J. 1991.** The effect of tributyltin upon steroid titres in the female dogwhelk, *Nucella lapillus,* and the development of imposex. *Marine Environnement Research*, **32**: 37-49.

**Stroben E., Oehlmann J. & Fioroni P. 1992.** The morphological expression of imposex in Hinia reticulata (Gastropoda: Buccinidae): a potential indicator of tributyltin pollution. *Marine Biology,* **113**: 625-636.

**Terlizzi A., Geraci S. & Gibbs P.E. 1999.** Tributyltin (TBT)-induced imposex in the Neogastropod *Hexaplex trunculus* in Italian coastal waters: morphological aspects and ecological implications. *Italian Journal of Zoology,* **66**: 141-146.

**Villa L., D'Agati P., Mansueto C., Pallerito C., Scopelliti M., Fiore T., Nagy L. & Pallerito L. 2003.** Effects of tributhyltin (IV) chloride on the gametes and fertilization of *Ascidia malaca* (Ascidiacea: Tunicata). *Applied in Organometallic Chemistry,* **17**: 107-112.