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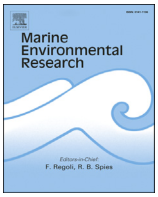

# Development of reproductive organs in the ivory shell *Babylonia japonica*: Observations from wild populations and laboratory-reared juveniles

Toshihiro Horiguchi [a,*], Yasuhiko Ohta [b], Fumihiko Hamada [c], Hiroshi Urushitani [a], Hyeon-Seo Cho [d], Hiroaki Shiraishi [a]

[a] *Center for Environmental Risk Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan*
[b] *Laboratory of Experimental Animals, Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori, Tottori 680-8553, Japan*
[c] *Tottori Prefectural Sea Farming Association, Tohaku-gun, Tottori 689-0602, Japan*
[d] *Faculty of Marine Technology, College of Ocean Science and Technology, Chonnam National University, San 96-1, Dundeok-dong, Yeosu 550-749, Republic of Korea*

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ABSTRACT

We histologically examined normal differentiation and development of genital tract and gonad in the ivory shell *Babylonia japonica* (Buccinidae) to determine whether the formation of male-type genitalia in imposex-exhibiting females mimics the normal development of male genitalia in prosobranch gastropods. We used a wild-caught 2-year-old specimen and laboratory-reared juveniles aged 0–24 months. Gonad differentiation was unclear before age 14 months, but progressed after 16 months. Both sexes had complete genital tract and mature gonad at 20 months. However, differentiation and development occurred earlier in females than in males. Development of genital tract preceded gonad differentiation. Vas deferens morphogenesis in males resembled that in imposex-exhibiting females. These findings help to understand the morphogenesis of genital tract and gonad in prosobranch gastropods and will contribute to more in-depth studies of the mode of action of organotin compounds such as TBT and TPhT in imposex development in female prosobranch gastropods.

## 1. Introduction

Masculinized female gastropod molluscs were first reported by Blaber (1970), who described a penis-like outgrowth behind the right tentacle in spent females of the dog-whelk, *Nucella lapillus*, around Plymouth, UK. The term "imposex," however, meaning "imposed sexual organs," was defined by Smith (1971) to describe the syndrome of superimposition of male genitalia, such as a penis and vas deferens, on female prosobranch gastropods. Imposex is thought to be an irreversible syndrome (Bryan et al., 1986). In severe cases of imposex, reproductive failure may occur, resulting in population decline or mass extinction (Gibbs and Bryan, 1986, 1996). Imposex in many species is induced by tributyltin (TBT) and triphenyltin (TPhT) released from antifouling paints on ships and fishing nets (Bryan et al., 1987, 1988; Gibbs et al., 1987; Horiguchi et al., 1995, 1997a). The use of TBT- or TPhT-based antifouling paints was addressed by the International Convention on the Control of Harmful Anti-fouling Systems on Ships (AFS Convention), which was adopted by the International Maritime Organization (IMO) on 5 October 2001 (IMO, 2001). According to the AFS Convention, all ships are prohibited from applying or re-applying organotin compounds as antifouling biocides after 1 January 2003, and by 1 January 2008 ships will either not have organotin compounds as antifouling biocides, or such antifouling systems will be covered with a coating that prevents leaching into the environment. It took longer than expected for the AFS Convention to be ratified by member states, but it finally came into effect on 17 September 2008 (http://www.imo.org/). Continued

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* Corresponding author.
*E-mail address:* thorigu@nies.go.jp (T. Horiguchi).

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monitoring is needed to protect marine and aquatic ecosystems from organotin pollution and to allow them to recover from its impacts.

As of 2005, approximately 200 species of prosobranch gastropods worldwide had been reported to be affected by imposex (Bech, 2002a,b; Fioroni et al., 1991; Horiguchi et al., 1997b; Marshall and Rajkumar, 2003; Shi et al., 2005; Sole et al., 1998; Ten Hallers-Tjabbes et al., 2003; Terlizzi et al., 2004). Many of these gastropods belong to the families Muricidae (e.g., *N. lapillus* and *Thais clavigera*), Buccinidae (e.g., *Babylonia japonica* and *Buccinum undatum*), and Nassariidae (e.g., *Ilyanassa obsoleta* and *Nassarius reticulatus*) of the Caenogastropoda (Fioroni et al., 1991; Horiguchi et al., 1997b). Many studies have examined the incidence or severity of imposex, investigated the use of certain gastropod species as biological indicators of TBT contamination, or surveyed TBT contamination by using gastropods. Only a few reports, however, have presented evidence for population-level effects of reproductive failure due to imposex, based on either morphology or histology (Bryan et al., 1986; Gibbs and Bryan, 1986; Gibbs et al., 1988, 1990, 1991; Horiguchi, 2000; Horiguchi et al., 1994, 2000, 2006; Oehlmann et al., 1996; Schulte-Oehlmann et al., 1997).

Six hypotheses have been proposed for the mechanisms by which organotins (i.e., TBT) induce imposex in prosobranch gastropods: (1) an increase in androgen (e.g., testosterone) levels as a result of TBT-mediated inhibition of aromatase (Bettin et al., 1996); (2) an increase in testosterone levels resulting from the inhibition of acyl CoA – steroid acyltransferase activity (Gooding et al., 2003; Sternberg and LeBlanc, 2006); (3) TBT-mediated inhibition of the excretion of androgen sulfate conjugates, with a consequent increase in androgen levels (Ronis and Mason, 1996); (4) TBT interference with the release of penis morphogenetic or retrogressive factors from the pedal or cerebropleural ganglia (Féral and Le Gall, 1983); (5) an increase in the level of an alanine–proline–glycine–tryptophan amide neuropeptide in response to TBT (Oberdörster and McClellan-Green, 2000); and (6) activation of the retinoid X receptor (RXR) (Nishikawa et al., 2004). Recent critical reviews of endocrinology and the mechanism of imposex caused by organotin compounds in prosobranch gastropods, however, have pointed out some contradictions associated with hypotheses 1 to 5 (Horiguchi, 2009; Scott, 2012, 2013). Consequently, hypothesis (6), suggesting activation of the RXR, seems plausible, because there are several studies that support this hypothesis without any contradictions (Castro et al., 2007; Horiguchi et al., 2007, 2008, 2010a,b; Sternberg et al., 2008; Urushitani et al., 2011). Therefore, we believe that RXR could be mediated molecular mechanisms of the differentiation, proliferation and morphogenesis of male genitalia in male and imposex-exhibiting female prosobranch gastropods.

The histological development of the vas deferens and penis in *N. lapillus* exhibiting imposex was reported by Gibbs and Bryan (1986) and Gibbs et al. (1987). Less is known about imposex development, in terms of the general development of the vas deferens and penis, in other species, although there are a few reports describing the general scheme of imposex development in prosobranch gastropods (e.g., Stroben et al., 1995). In the case of *T. clavigera*, this process remained unclear until recently, because extensive contamination by organotins (TBT and TPhT) throughout Japan (Horiguchi et al., 1994) meant that only specimens in the severe stages of imposex had been observed there. Horiguchi et al. (2012), by histological analysis of less-severely exposed specimens from a wild population and females exposed to TBT in the laboratory, finally revealed the steps in the development of the vas deferens and penis in the imposex-exhibiting female rock shell *T. clavigera*. They observed a variety of patterns of vas deferens morphogenesis in wild females of *T. clavigera*. The immature vas deferens, however, was observed only beneath or behind the penis, and no vas deferens was observed close to the vaginal opening (i.e., vulva) of the capsule gland in TBT-exposed female *T. clavigera*. This differed from the vas deferens formation observed in wild female *T. clavigera*, as well as in female *N. lapillus* (Gibbs et al., 1987). Considering the observations of both wild female *T. clavigera* and TBT-exposed females in the laboratory, the vas deferens sequence (VDS) index for *T. clavigera* was proposed as VDS 1–6 (Horiguchi et al., 2012). This VDS index differs from that of *N. lapillus* (Horiguchi et al., 2012), especially in the initial developmental stages of imposex. Thus, it is possible that the processes responsible for development of the vas deferens and penis in imposex-exhibiting female gastropods differ among gastropod species.

In the ivory shell *B. japonica*, histopathological and analytical chemical studies strongly suggest that reproductive failure in adult females accompanying imposex, possibly induced by TBT and TPhT, could have caused a marked decline in *B. japonica* populations; this decline might be one factor behind the decrease in the total catch of *B. japonica* in Japan since the 1970s (Horiguchi et al., 2006). Here, we examined female and male *B. japonica* to discern normal differentiation and development of the genital tract and gonad; this will be useful in determining whether the formation of male-type genitalia in imposex-exhibiting females mimics the normal development of male genitalia in prosobranch gastropods.

## 2. Materials and methods

### 2.1. *B. japonica* specimen from the wild population in Miho Bay, Tottori, Japan

A 2-year-old male *B. japonica*, which had been produced as seed at the hatchery of the Tottori Prefectural Sea Farming Association (TPSFA) and then released, was captured in trawling nets in Miho Bay, Japan (35°27′50.37″ N, 133°21′01.84″ E: Fig. 1) in July 2010. The TPSFA hatchery in Tomari, Japan, has been conducting artificial production, culture and release of *B. japonica* as seed or juveniles since the 1980s to enhance the population of this species in Tottori Prefecture, Japan (Kajikawa et al., 1983; Kajikawa, 1984). This captured specimen was one such produced.

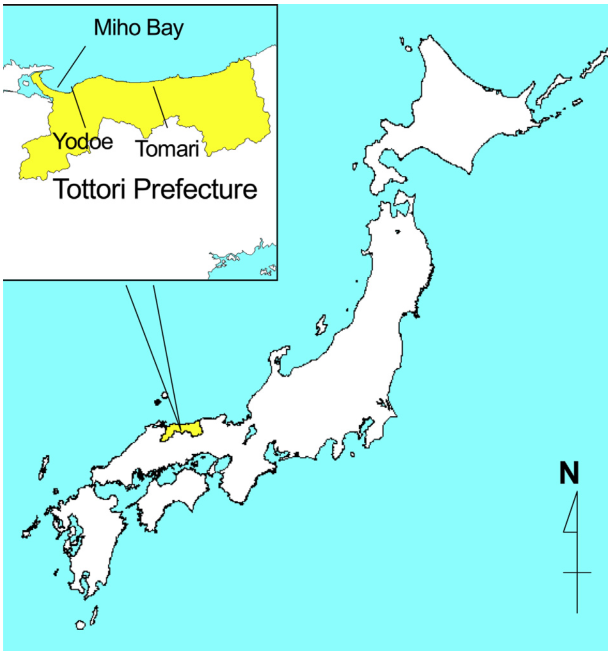

**Fig. 1.** Sampling sites for specimens of the ivory shell *Babylonia japonica*.