

# Continuing observation of disturbed reproductive cycle and ovarian spermatogenesis in the giant abalone, *Haliotis madaka* from an organotin-contaminated site of Japan

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## Abstract

Histological examination of gonads as well as chemical analysis of organotin compounds in tissues of the giant abalone, *Haliotis madaka*, was conducted to evaluate continuing endocrine disruption in abalone populations in Japan. Abalone specimens were collected from two different areas, Tsushima as a reference site and Jogashima as a site representative of declining abalone populations where serious organotin contamination had been observed, each month from January 1998 to March 1999. Scores were given to the development stages of reproductive cells in the ovary and testis. The degree of sexual maturation was evaluated by calculating the mean value of a histogram of these scores for the reproductive cells of each abalone. The temporal variations in degree of sexual maturation showed that female and male abalone from Tsushima matured synchronously, while those from Jogashima did not, which were similar to results of the previous study during September 1995–November 1996. Approximately 19% of the female abalone from Jogashima were masculinized with an ovo-testis, which was also similar to the result of the previous study. The masculinization of female abalone is reported to be induced by tributyltin (TBT) and triphenyltin (TPhT) from antifouling paints. Concentrations of the sum of butyltins (TBT, dibutyltin (DBT) and monobutyltin (MBT):  $\Sigma$ BTs) and the sum of phenyltins (TPhT, diphenyltin (DPhT) and monophenyltin (MPhT):  $\Sigma$ PhTs) in the muscle of abalone from Jogashima ( $n = 73$ ) of  $7.8 \pm 9.0$  ng/g wet wt. and  $4.5 \pm 6.8$  ng/g wet wt., respectively, were significantly higher than those from Tsushima ( $n = 87$ ) of  $4.7 \pm 4.9$  ng/g wet wt. and  $0.8 \pm 1.7$  ng/g wet wt., respectively ( $p < 0.05$  for  $\Sigma$ BTs;  $p < 0.001$  for  $\Sigma$ PhTs), although concentrations of TBT and TPhT in the muscle of abalone from Jogashima ( $n = 73$ ) of  $2.2 \pm 2.5$  ng/g wet wt. and  $5.8 \pm 5.1$  ng/g wet wt., respectively, were insignificantly and significantly higher than those from Tsushima ( $n = 87$ ) of  $0.4 \pm 0.6$  ng/g wet wt. and  $0.5 \pm 0.9$  ng/g wet wt., respectively, ( $p > 0.05$  for TBT;  $p < 0.001$  for TPhT). Thus, endocrine disruption as well as contamination by organotins in the giant abalone from Jogashima is still persisting. © 2005 Elsevier Ltd. All rights reserved.

**Keywords:** Endocrine disruption; Reproductive cycle; Ovarian spermatogenesis; Abalone; Tributyltin (TBT); Triphenyltin (TPhT)

## 1. Introduction

A remarkable population decline has been observed in Japanese abalone since the 1970s, although much

effort (e.g. artificial production and release of juvenile abalone into the sea) has been made to enhance the abalone stocks (Imai, 1996). The proportion of artificially released individuals, which are distinguishable from natural stocks by the green color of the tips of the shells, has exceeded 95% of the total abalone captured in some areas, such as Jogashima (Kanagawa Prefecture) (Imai, 1996). This suggests that reproduction in natural abalone stocks is declining.

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Reduced abalone recruitment may result from several factors, including mass mortality of larvae and/or juveniles (due to sudden large changes in seawater temperature, food availability, increased predation and/or increased incidence of disease), reduced egg production, low fertilization rate (possibly due to pollutants in the marine environment) and/or overfishing (by commercial fishery) (Takiguchi, 2002). The causal factors for such population declines in abalone have been investigated, but are still unknown.

Imposex is the superimposition of male sexual organs in female gastropod molluscs, which brings about reproductive failure in severely affected individuals (Smith, 1971; Gibbs and Bryan, 1986). Imposex is considered to be an endocrine disruption in gastropods, which is typically induced by tributyltin (TBT) and triphenyltin (TPhT) from antifouling paints (Gibbs et al., 1987; Horiguchi et al., 1997a).

The areas where marked decreases in abalone populations occurred broadly correspond to sites contaminated with organotin compounds (TBT and TPhT), and the time period of this decrease broadly corresponds to a history of marine pollution by organotins. Therefore, we have been investigating a possible link. Our hypothesis is that organotins cause endocrine disruption in abalone, in a similar way to the induction of imposex in neogastropods, and that this factor might contribute to decreases in abalone populations.

Our previous studies have shown that (1) ovo-testis (approximately 20% of collected female specimens) as well as disturbed reproductive cycles have been observed in the giant abalone, *Haliotis madaka* and also the related species, *H. gigantea* from a site of known population decline, Jogashima, (2) tissue concentrations of organotins, such as TBT and TPhT, were significantly higher in the abalone specimens from Jogashima compared to control specimens from a reference site, Tsushima ( $p < 0.01$ ), (3) in situ exposure of abalone from Tsushima caged near a dockyard in Jogashima for seven months resulted in spermatogenesis in the ovaries of approximately 90% of females, as well as a significant tissue accumulation of TBT and TPhT, and (4) ovarian spermatogenesis was observed in *H. gigantea* exposed to TBT and TPhT for two months in a flow-through exposure system in the laboratory (Horiguchi et al., 2000, 2002).

The objective of this study was to verify whether disturbed reproductive cycles as well as ovarian spermatogenesis were still observed in the giant abalone, *H. madaka* from Jogashima over a period of 1998 and 1999.

## 2. Materials and methods

A total of 15 *H. madaka* (giant abalone) individuals more than 10 cm in length were collected monthly from Tsushima, Nagasaki Prefecture, as a reference site, and

Jogashima, Kanagawa Prefecture, as a site representative of areas where abalone populations have declined drastically, from January 1998 to March 1999. In Tsushima, abalone stocks are relatively stable and contamination levels of organotin compounds, such as TBT and TPhT, are very low (Horiguchi et al., 1997b, 2000). Jogashima was known to be heavily contaminated with organotins, following an imposex survey there of the rock shell, *Thais clavigera*, and is one of the most contaminated sites in Japan with regard to TBT and TPhT (Horiguchi et al., 1997b, 2000).

Gonad samples for histological examination were fixed in Gendre's fluid (a 15:5:1 solution of saturated 2,4,6-trinitrophenol in 30% ethanol:35% formaldehyde:acetic acid), embedded in paraffin, stained with hematoxylin–eosin (HE) and observed with a light microscope. Scores for the development of reproductive cells were applied to evaluate quantitatively the gonadal maturation of the abalone, using the developmental stages described in Tomita (1967, 1968) (Horiguchi et al., 2000). The individual reproductive developmental score was defined as the mean value of a histogram of these scores for the reproductive cells of each abalone. The population reproductive developmental score was defined as the monthly mean value of the individual reproductive developmental scores (Horiguchi et al., 2000).

Chemical analysis of organotin compounds (TBT, dibutyltin (DBT), monobutyltin (MBT), TPhT, diphenyltin (DPhT) and monophenyltin (MPhT)) in muscle tissue of each abalone specimen was conducted according to the method described in Horiguchi et al. (1994). Composite tissues of ctenidium, hypobranchial gland, radula, esophagus, stomach, digestive gland, rectum, testis, ovary and the head including central nervous system ganglia were also used for chemical analysis of organotins. Briefly, tissues were extracted with 0.1% tropolone/benzene and 1 N HBr/ethanol by ultrasonication, derivatized with propylmagnesium bromide, cleaned by silica gel column chromatography and quantified by gas chromatography with a flame photometric detection. The detection limit of the instrument was 50 pg, and certified reference material of Japanese sea bass, *Lateolabrax japonicus*, for TBT and TPhT analysis (prepared by National Institute for Environmental Studies, NIES CRM no. 11) was used for quality assurance and quality control. The analytical conditions are described in more detail in Horiguchi et al. (1994).

The results of this study were tested statistically by analysis of variance (ANOVA) with two-sample testing for normal distribution with equal variances (Welch's test).

## 3. Results and discussion

Temporal variations in the reproductive developmental score of the populations still differed between the two

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