Chapter 8
The Endocrine-Disrupting Effect of Organotin Compounds for Aquatic Organisms

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Abbreviations CRM: Certified Reference Material; DBT: Dibutyltin; DPT: Diphenyltin; GC-FPD: Gas chromatography with flame photometric detection; AFS Convention: International Convention on the Control of Harmful Anti-fouling System on Ships; IMO: International Maritime Organization; MBT: Monobutyltin; MPT: Monophenyltin; RPL: Relative penis length; ΣBTs: Sum of butyltins; ΣPTs: Sum of phenyltins; TBT: Tributyltin; TPT: Triphenyltin; VDS: Vas deferens sequence; wet wt: Wet weight

8.1 Introduction

The first report of masculinized female gastropod mollusks was made by Blaber (1970), describing a penis-like outgrowth behind the right tentacle in spent females of the dog-whelk, *Nucella lapillus* around Plymouth, UK. The term, "imposex", however, was defined by Smith (1971), meaning imposed sexual organs, to describe the syndrome of a superimposition of male genital tracts, such as penis and vas deferens, on female gastropods. Imposex is thought to be an irreversible syndrome (Bryan et al. 1986). Reproductive failure may be brought about in severely affected stages of imposex, resulting in population decline and/or mass extinction (Gibbs and Bryan 1986, 1996). Imposex is known to be induced in many species by tributyltin (TBT), and also by triphenyltin (TPT) released from antifouling paints on ships and fishing nets (Bryan et al. 1987, 1988; Gibbs et al. 1987; Horiguchi et al. 1995, 1997a).

Up until July 2004, approximately 150 gastropod species worldwide have been reported to be affected by imposex (Bech 2002a, b; Fioroni et al. 1991; Horiguchi et al. 1997b; Marshall and Rajkumar 2003; Sole et al. 1998; ten Hallers-Tjabbes et al. 2003;

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Terlizzi et al. 2004). Regarding Japanese gastropods, 39 species, including seven mesogastropods and 32 neogastropods, have been found to be affected by imposex (Horiguchi 2000). Several of these belong to the genus Muricidae (e.g., *Lepsiella scobina, Nucella lapillus*, *N. lima*, *Ocenebra erinacea*, *Ocinebrina aciculata*, *Rapana venosa venosa*, *Thais clavigera*, *Urosalpinx cinerea* etc.); Buccinidae (e.g., *Babylonia japonica*, *Buccinum undatum*, *Neptunea arthritica arthritica* etc.); Conidae (e.g., *Conus marmoreus bandanus*, *Virroconus ebraeus* etc.) and Nassariidae (e.g., *Ilyanassa obsoleta*, *Hinia reticulata* etc.). Numerous studies have examined the incidence or severity of imposex; investigated the use of certain gastropod species as biological indicators of TBT contamination, and surveyed TBT contamination also using gastropods. Only a few reports, however, have presented evidence for population-level effects of reproductive failure due to imposex, based on either morphological or histological methods (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).

Here, the author will summarize current status of imposex and contamination by organotins in the rock shell *Thais clavigera* in Japan, and then focus on two case studies of imposex in the ivory shell *Babylonia japonica* and masculinization of female abalone *Haliotis madaka* and *H. gigantea*, which is similar to imposex in other gastropods (Meso- and Neogastropoda), with special reference to possible linkages with declining populations.

8.2 Current Status of Imposex and Contamination by Organotins in the Rock Shell *Thais clavigera* from Japan

Among rock shell (*T. clavigera*) samples collected between January 1999 and November 2001 from 174 locations along the Japan coast, imposex was observed at 166 locations, whereas no, or rare, cases were found at the remaining eight locations. The percentage occurrence of imposex was as high as or close to 100% in approximately half of the affected locations surveyed. It is expected that spawning obstruction occurs in more than half the population of females when relative penis length (RPL) index exceeds 40, on the basis of the relationship between RPL index, vas deferens sequence (VDS) index and the percentage occurrence of oviduct (vulva) blockage in females. Among the 174 locations, RPL index values exceeding 40 were found in 41 locations. High values of RPL and VDS indices were generally observed in the western part of Japan. Compared with the results of a previous survey (conducted between 1996 and 1999), the indices seemed to have decreased, but remained almost unchanged in some locations (Horiguchi 2004).

TPT concentrations in tissues of the rock shell showed a decrease over time but varied distinctly between locations; relatively high pollution levels in a few locations were detected. Decreases in TBT concentrations were also distinct in general but the degree of decrease was lower than those in TPT concentrations.

Changes in concentrations over time were not observed in several locations. An increase in the concentrations of TBT was observed in two locations near fishing ports (Horiguchi 2004).

8.3 Collapse of Commercial Fisheries for the Ivory Shell *Babylonia japonica* in Japan: Did Reproductive Failure Caused by Imposex Bring About Drastic Population Decline?

The ivory shell *Babylonia japonica* (Neogastropoda: Buccinidae), which inhabits sandy or muddy sediments in shallow water (approximately 10–20 m in depth) from the south of Hokkaido to Kyushu, Japan, is a scavenger in inshore ecosystem, and traditionally a target species of commercial fisheries in Japan. Imposex seems to have been observed in the ivory shell since the 1970s (Kajikawa and Hamada, personal communication, Tottori, Japan, July 1991), and the total catch drastically decreased all over Japan in the late 1970s or early 1980s (Horiguchi and Shimizu 1992).

Much effort has been made to enhance the ivory shell stocks: Seed production using adult ivory shells reared in hatcheries, with subsequent release of seeds/juveniles into the sea. Most of seeds/juveniles of the ivory shells released into the sea (approximately 90% of total production in Japan) have been produced at a hatchery in Tomari, Tottori Prefecture, located in the western part of Japan (Horiguchi et al. 2006). In Tottori Prefecture, however, not only the total catch but also the number of egg capsules spawned by adult shells at the hatchery and seeds/juveniles artificially produced/released into the sea has decreased since the mid-1980s (Horiguchi et al. 2006; Fig. 8.1). The total catch has drastically decreased since 1984, 2 years after the first observation of imposex-affected female ivory shells from Tottori Prefecture, involving the increase of both percentage occurrence of imposex individuals and mean penis length in females (Hamada et al. 1988, 1989; Kajikawa 1984; Kajikawa et al. 1983; Fig. 8.1). The number of egg capsules spawned by adult ivory shells at the hatchery, as well as the number of seeds/juveniles artificially released into the sea, has also decreased since the mid-1980s (Fig. 8.1). Introduction of adult ivory shells from another prefecture (Niigata Prefecture, Japan) to compensate for insufficient numbers of the normal brood stock also resulted in failure of the release of seeds/juveniles into the sea due to their high mortality at the hatchery prior to release (Fig. 8.1). Recovery of total catch of the ivory shell has not been observed in spite of such efforts to enhance the ivory shell stocks (Fig. 8.1). Finally, operation of the ivory shell hatchery for stock enhancement in Tottori had to be stopped, and the hatchery was closed in 1996 (Fig. 8.1). Therefore, possible reproductive failure caused by imposex in the ivory shell was suspected.

Horiguchi et al. (2006) examined the incidence of reproductive failure accompanied by imposex in the ivory shell, based on the histopathological observation of gonads, and investigated the relationship between organotin compounds and imposex in the ivory shell, based on chemical analysis of organotin concentrations

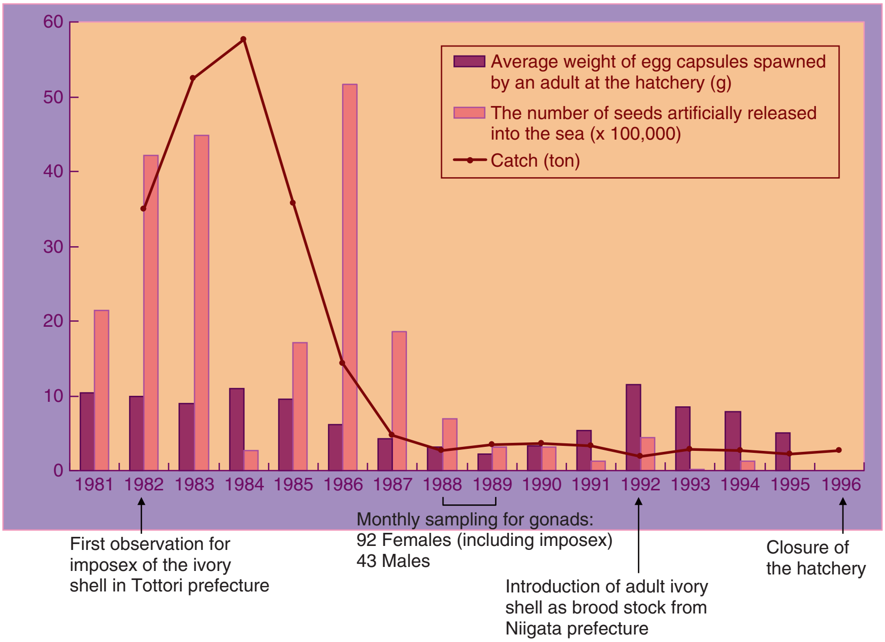

Fig. 8.1 Temporal trends for average weight of egg capsules spawned by adults at the hatchery, the number of seeds/juveniles released into the sea and the total catch of the ivory shell, *Babylonia japonica* in Tottori Prefecture, Japan (Horiguchi et al. 2006)

in tissues of the ivory shell. Horiguchi et al. (2006) also discussed the possibility that the marked decline in the ivory shell (*Babylonia japonica*) populations from Japan could have been brought about mainly by reproductive failure accompanied by imposex, induced by TBT and TPT from antifouling paints.

Horiguchi et al. (2006) performed histopathological examination of gonads in the ivory shell: Adult *B. japonica* reared in the hatchery of the Tottori Prefectural Sea Farming Association were sampled monthly from December 1988 to November 1989, when the number of egg capsules spawned by adult *B. japonica* at the hatchery had reached a minimum. Gonad samples of 10–15 *B. japonica* specimens were fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin-eosin for histopathological examination under a light microscope. In total, 135 *B. japonica* specimens were examined (43 males and 92 females, consisting of 16 normal females and 76 imposex-exhibiting individuals). To quantitatively evaluate the gonadal maturation of *B. japonica,* female and male reproductive cells were scored based on developmental stages, similar to those described in Takamaru and Fuji (1981) and Horiguchi et al. (2000). The individual reproductive developmental score was the mean value of these scores for the reproductive cells of each *B. japonica*. The population reproductive developmental score was the monthly mean value of the individual reproductive developmental scores (Horiguchi et al. 2000).

Horiguchi et al. (2006) also carried out chemical analysis of organotin compounds in tissues of the ivory shell: Adult *B. japonica* specimens collected at Yodoe, Tottori

Prefecture, in June 1991 were used for chemical analysis of organotin (butyltin and phenyltin) compounds. The specimens were dissected for original sex and imposex determination. A total of 52 *B. japonica* specimens were used for chemical analyses (25 males and 27 females, consisting of three normal females and 24 imposex-exhibiting individuals). Chemical analyses of butyltin and phenyltin compounds in tissues (muscle [foot], head with tentacle, radula with sac, oesophagus with crop, stomach, digestive gland, kidney, rectum, ovary or testis, oviduct, siphon, ctenidium, heart, osphradium, and mantle) of *B. japonica* specimens were conducted with composite samples and quantified by gas chromatography with flame photometric detection (GC-FPD), using the method described in Horiguchi et al. (1994).

The percentages of occurrence of imposex were 82.6% and 88.9% in *B. japonica* specimens collected from December 1988 to November 1989 and in June 1991, respectively. Both penis and vas deferens were found to be well developed in imposex-exhibiting females (Horiguchi et al. 2006). No oviduct blockage (i.e., occlusion of the vulva) by vas deferens formation, however, was observed in imposex-exhibiting female *B. japonica* (Horiguchi et al. 2006), a finding that differs from the imposex symptoms observed in *N. lapillus*, *Ocinebrina aciculata*, and *T. clavigera* (Gibbs and Bryan 1986; Gibbs et al. 1987; Horiguchi et al. 1994; Oehlmann et al. 1996).

Temporal variations in the reproductive developmental score of the *B. japonica* population differed between females (including imposex-exhibiting females) and males (Horiguchi et al. 2006; Fig. 8.2). Although the spawning season for *B. japonica*

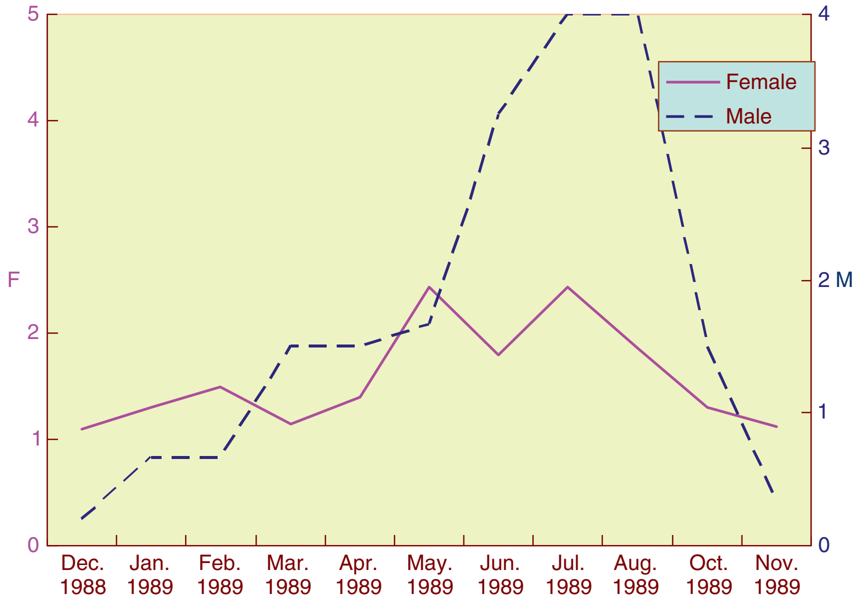

Fig. 8.2 Reproductive cycle of the ivory shell (*Babylonia japonica*) represented by the population reproductive developmental scores. Female reproductive cells were scored based on five categories, and those of males were based on four categories (Horiguchi et al. 2006). The female curve includes imposex-exhibiting females

is late June to early August (Kajikawa et al. 1983), ovarian maturation seemed to be suppressed in females, compared to testicular maturation in males (Horiguchi et al. 2006; Fig. 8.2), which is probably due to the presence of immature females throughout the spawning season. During the spawning season, clearer ovarian maturation and spawning of many more egg capsules were observed in *B. japonica* females in a population from Teradomari, Niigata Prefecture, Japan, compared to those from Tottori (Hamada and Inoue 1993, 1994, 1995). Testicular maturation in males from Tottori was clear in July and August, the spawning season for *B. japonica* (Horiguchi et al. 2006; Fig. 8.2). Thus, the reproductive cycle was unclear in females but it was clearly observed in males (Horiguchi et al. 2006; Fig. 8.2). This suppressed ovarian maturation during the spawning season could be the direct reason for the decreased number of egg capsules spawned by adult *B. japonica* at the hatchery and might accompany imposex in *B. japonica* (Gibbs et al. 1988).

Ovarian spermatogenesis (i.e., an ovo-testis) was observed in 6 (one normal female and five imposex individuals) of 92 female or imposex *B. japonica* specimens examined, a frequency of about 6.5% (Horiguchi et al. 2006; Fig. 8.3). It is well known that most prosobranchs (including *B. japonica*) are dioecious although there are relatively few hermaphroditic prosobranchs in which the gonad produces eggs and sperm simultaneously (Fretter 1984; Uki 1989). Ovarian spermatogenesis has been observed in neogastropods (e.g., *N. lapillus*, *O. aciculata,* and *T. clavigera*) and archaeogastropods (e.g., *Haliotis madaka* and *H. gigantea*) exposed to TBT or TPT, although no penis formation is involved in spermatogenesis in ovaries of female abalone (see below) (Gibbs et al. 1988; Horiguchi and Shimizu 1992; Horiguchi et al. 2000, 2002, 2005; Oehlmann et al. 1996). Ovarian spermatogenesis was even observed in a normal female *B. japonica* without any penis or vas deferens formation, although the frequency was low (one of six, 16.7%). The development of male-type genital organs (penis and vas deferens) and ovarian spermatogenesis in females exposed to TBT or TPT might be controlled through different physiological pathways. This ovarian spermatogenesis may be one of the reasons why the spawning ability of female *B. japonica* decreased (Horiguchi et al. 2006).

Tissue concentrations of organotin compounds, such as butyltins and phenyltins, were determined by GC-FPD, and different tissue distributions were observed (Horiguchi et al. 2006; Fig. 8.4). A marked accumulation of TBT was observed in the ctenidium, osphradium, and heart in both males and females, whereas the highest concentrations of TPT were detected in the ovaries of females and the digestive glands of males (Horiguchi et al. 2006; Fig. 8.4). Based on the total body burden of TBT in *B. japonica*, more than one-third of total TBT accumulated in the digestive glands of both males and females, followed by the testis, ctenidium, muscle, and heart in males and the muscle, ovary, ctenidium, and head (including the central nervous system ganglia) in females (Horiguchi et al. 2006; Figs. 8.5a, c). Based on the total body burden of TPT, approximately three-quarters and more than one-half of total TPT accumulated in the digestive glands of males and females, respectively. The second highest tissue burden of TPT was observed in the gonads of both males and females, followed by the muscle, ctenidium, and heart in males and the muscle, oviduct, and head in females (Horiguchi et al. 2006; Figs. 8.5b, d).

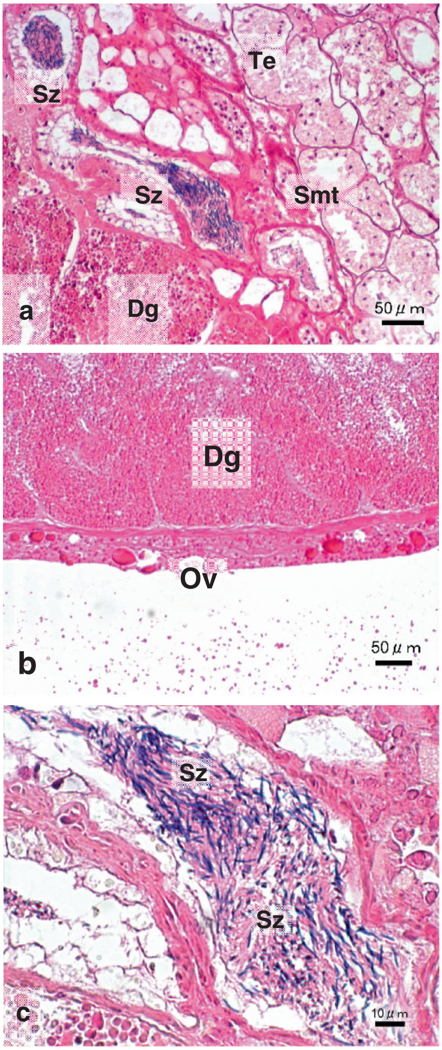

Fig. 8.3 Spermatogenesis in the ovary of a normal female *Babylonia japonica* (i.e., without penis and vas deferens). Testicular (a) and ovarian (b) tissues (i.e., ovo-testis) were observed in the gonad of a female *B. japonica*, which was classified originally as a female because of the presence of female accessory sex organs (e.g., a capsule gland) with neither penis nor vas deferens. Spermatogenesis was also observed in seminiferous tubules of the ovo-testis (c). Dg, digestive gland; Ov, ovary; Smt, seminiferous tubule; Sz, spermatozoon; Te, testis (Horiguchi et al. 2006)

A similar accumulation pattern was also observed in *T. clavigera* (Horiguchi et al. 2003), whereas a slightly different pattern was found in *O. erinacea*, in which approximately half of the total body TBT burden accumulated in the capsule gland (Gibbs et al. 1990), possibly suggesting a difference in organotin accumulation patterns among species. Although concentrations of TBT and TPT in ganglia were quite high in *T. clavigera*, the total tissue burden of those organotins was not high because of the relatively small ganglia tissue in that species (Horiguchi et al. 2003); this may also be the case with *B. japonica* in this study. Similar concentrations of TBT and TPT were also detected in ganglia of *B. undatum* (Mensink et al. 1997).

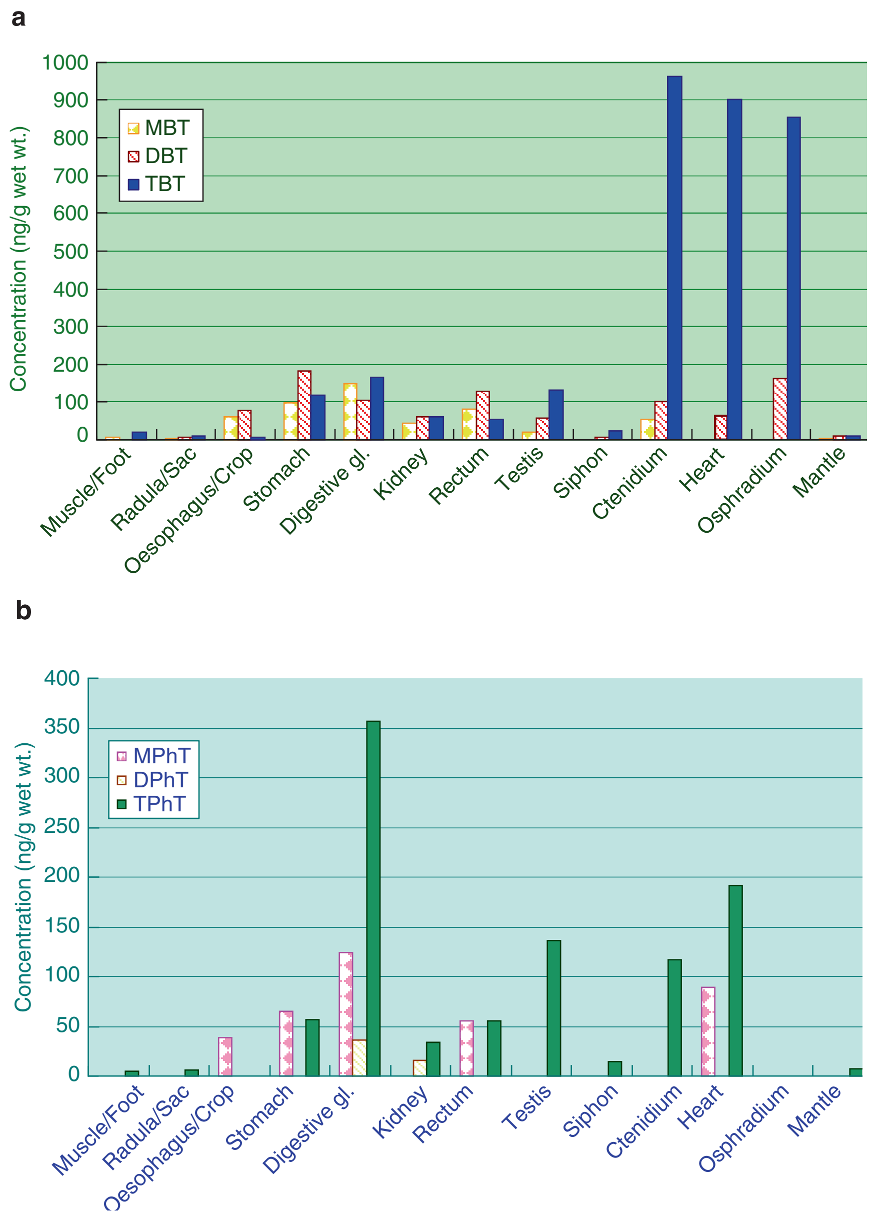

Fig. 8.4 Tissue distribution of organotin compounds in the ivory shell (*Babylonia japonica*) from Yodoe, Tottori, Japan (June 1991): (**a**) butyltins in males; (**b**) phenyltins in males;

Mortality of larvae and seeds or juveniles might also be due to the accumulation of TPT and TBT in ovaries as well as contamination of seawater with TPT or TBT (Coelho et al. 2001; Inoue et al. 2004; Lapota et al. 1993; Li et al. 1997; Nakayama et al. 2005; Ruiz et al. 1995; Treuner et al., unpublished manuscript). Based on a

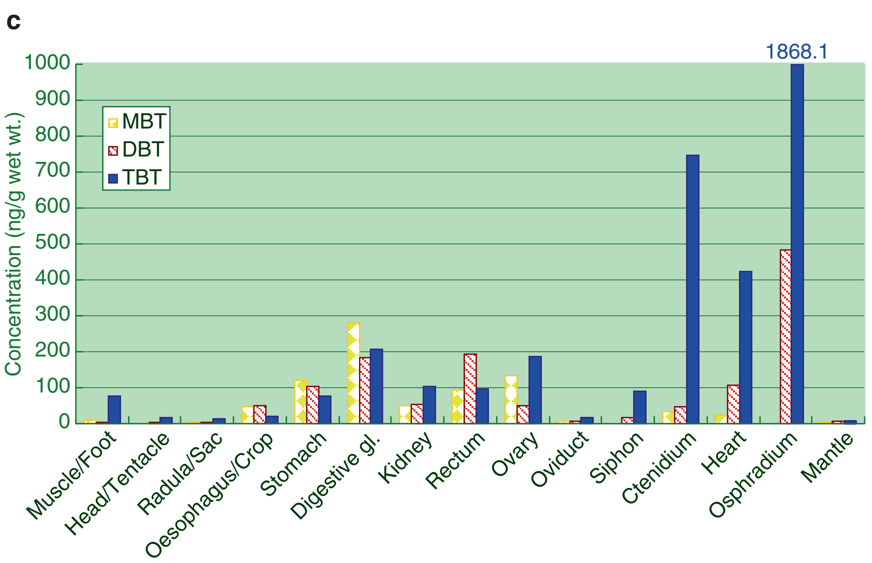

Fig. 8.4 (continued) (**c**) butyltins in females (including imposex individuals);

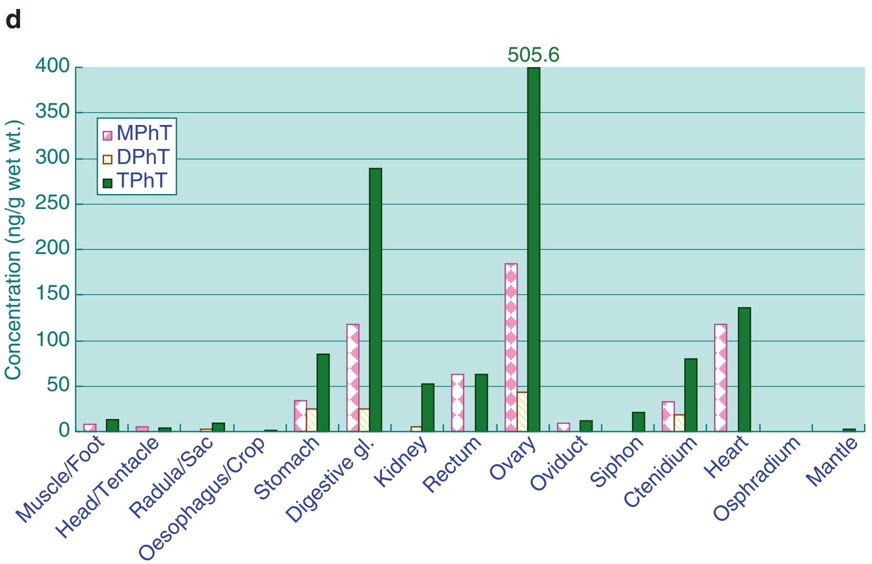

Fig. 8.4 (continued) (**d**) phenyltins in females (including imposex individuals) (Horiguchi et al. 2006)

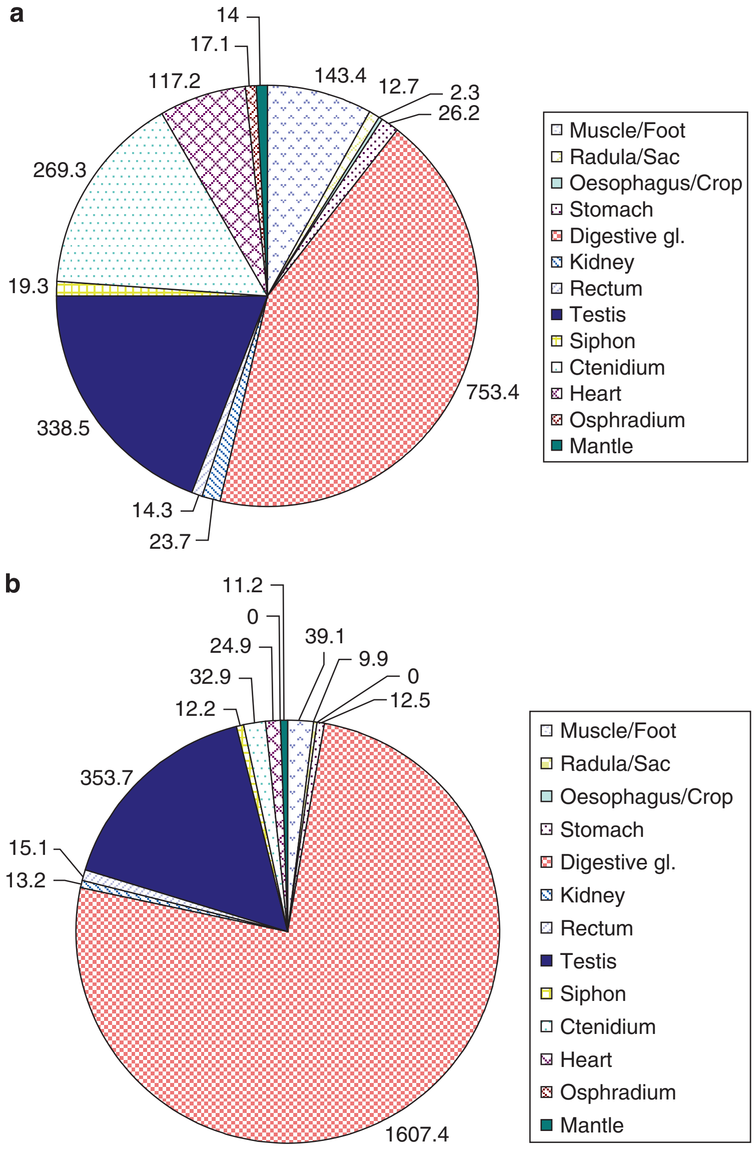

Fig. 8.5 Total body burden of organotin compounds in the ivory shell (*Babylonia japonica*) from Yodoe, Tottori, Japan (June 1991) (unit: ng): (**a**) tributyltin in males; (**b**) triphenyltin in males;

survey of imposex and organotin concentrations in tissues of *T. clavigera* (Horiguchi et al. 1994), contamination with TBT and TPT was relatively high along the coast of Tottori Prefecture, especially in Miho Bay, where the *B. japonica* specimens used in this study were collected.

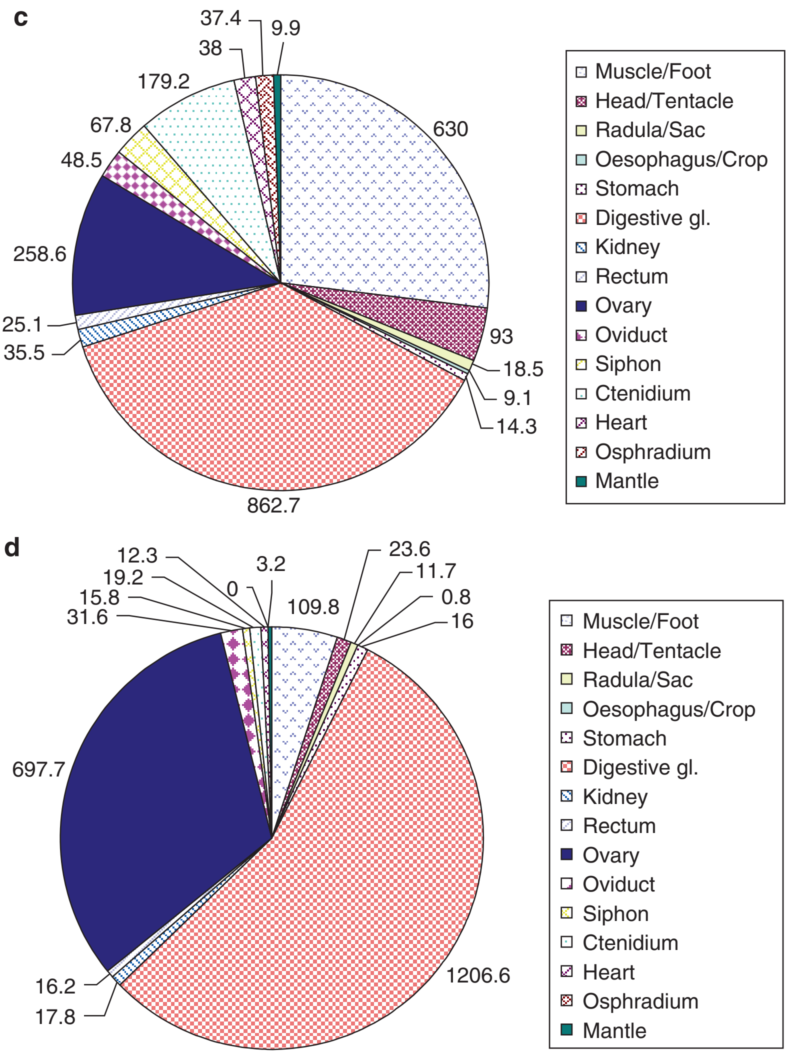

Fig. 8.5 (continued) (**c**) tributyltin in females (including imposex individuals); (**d**) triphenyltin in females (including imposex individuals) (Horiguchi et al. 2006)

Concentrations of TBT and TPT were relatively high in the ovaries of females (Horiguchi et al. 2006; Fig. 8.4). Both TBT and TPT concentrations in gonads were positively correlated with penis length in females (Horiguchi et al. 2006; Fig. 8.6), as was the case with *T. clavigera* (Horiguchi et al. 1994; Shim et al. 2000). Laboratory experiments revealed that both TBT and TPT induced or promoted the development of imposex in *T. clavigera* (Horiguchi et al. 1995, 1997a); therefore, imposex could be caused by TBT or TPT in *B. japonica* as well. However, it is difficult to estimate the threshold concentration of TBT and/or TPT that induces

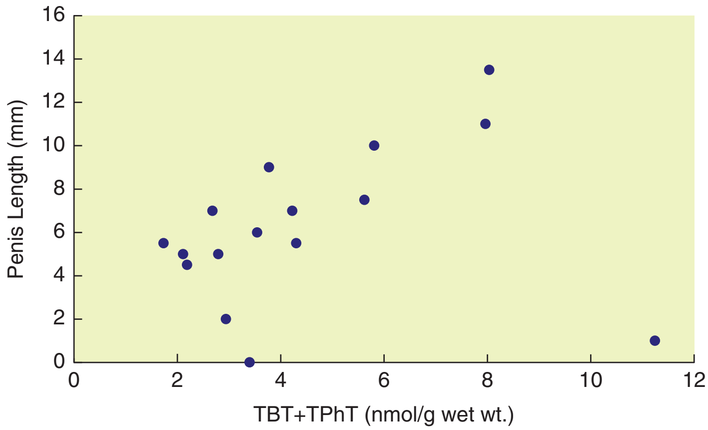

Fig. 8.6 Relationship between triorganotin (the sum of tributyltin and triphenyltin) concentrations in gonads and penis length in female *Babylonia japonica* (Horiguchi et al. 2006)

the development of imposex in *B. japonica*. Laboratory flow-through exposure experiments with *B. japonica*, using TBT and TPT, are needed to estimate the threshold concentration for the development of imposex. The estimated threshold concentration of TBT (in whole body tissues) inducing the development of imposex was reported to be approximately 20 ng Sn/g dry weight (corresponding to approximately 10–12.5 ng TBT/g wet weight, supposing that the concentration on a dry weight basis is 4–5 times that on a wet weight basis) for *N. lapillus* (Gibbs et al. 1987), and to be 10–20 ng/g wet weight for *T. clavigera* (Horiguchi et al. 1994). Because of limited experimental and analytical data for *B. japonica*, however, it is difficult to compare the sensitivity to TBT and TPT between this and other gastropod species, such as *N. lapillus*, *Ocenebra erinacea, Urosalpinx cinerea* and *T. clavigera* (Bryan et al. 1987; Gibbs et al. 1987, 1990, 1991; Horiguchi et al. 1994, 1995).

The planktonic stage of *B. japonica* is estimated to last approximately 4–5 days (Hamada et al. 1988, 1989). This means the recruitment of veliger larvae from other populations inhabiting remote, less contaminated areas is unlikely. Reproductive failure accompanied by imposex in females could result in extirpation of the *B. japonica* population within several years, because the number of offspring produced by adult *B. japonica* in the population is likely to continue to decrease. The existence and duration of a free-swimming phase during larval development is an important factor in determining the linkage between impaired reproductive ability, caused by imposex, to population decline (Bryan et al. 1986; Gibbs and Bryan 1986; Gibbs et al. 1988, 1990, 1991; Horiguchi et al. 2006).

In conclusion, it is suggested that reproductive failure (suppressed ovarian maturation and ovarian spermatogenesis) in adult females with imposex, possibly induced by TBT or TPT from antifouling paints, could have brought about the marked decline in *B. japonica* populations that has been observed.

8.4 Could Ovo-Testis and a Disturbed Reproductive Cycle in the Giant Abalone *Haliotis madaka* be Linked with Organotin Contamination in a Site of Population Decline?

A remarkable population decline has been observed in Japanese abalone since the 1970s (Fig. 8.7), although much effort (e.g. artificial production and release of juvenile abalone into the sea) has been made to enhance stocks (Imai et al. 2006). 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 et al. 2006). This suggests that reproduction in natural abalone stocks is declining.

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). The causal factors for such population declines in abalone have been sought, but are still unknown (Imai et al. 2006).

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

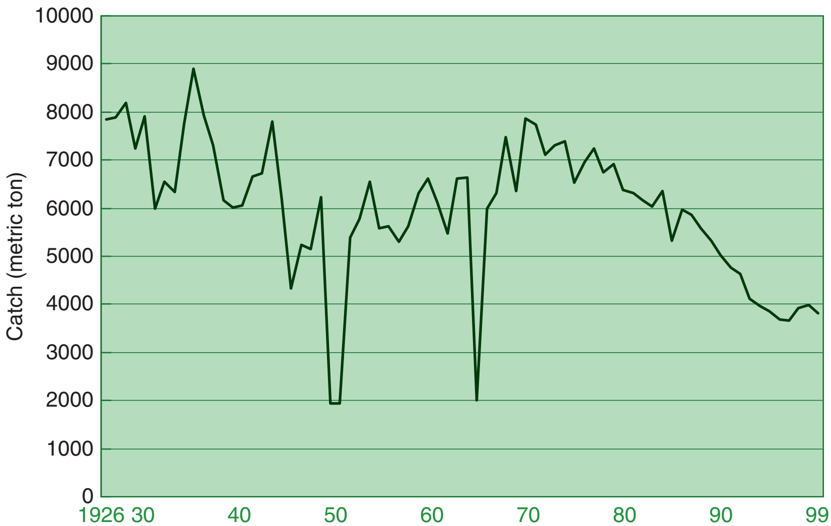

Fig. 8.7 Temporal trend of the total catch of abalone in Japan

The areas where abalone populations have decreased remarkably and the period when this occurred correspond broadly to sites contaminated with organotin compounds and sites with a history of marine pollution by organotins, respectively. Therefore, it is hypothesized that endocrine disruption in abalone has been caused by organotins, and has contributed to population decline (Horiguchi et al. 2000).

A total of 15 *Haliotis madaka* (giant abalone) individuals more than 10 cm in length were collected monthly from two sites between September 1995 and November 1996. The first site, at Tsushima, Nagasaki Prefecture, was a reference site, while the second, at Jogashima, Kanagawa Prefecture, was representative of areas where abalone populations have declined drastically. In Tsushima, abalone stocks are relatively stable and contamination levels of organotin compounds, such as TBT and TPT, are very low (Horiguchi et al. 1997b). 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 TPT (Horiguchi et al. 1997b). The abalone specimens were divided into two groups: more than half of the gonad samples were used for histological examination and the rest for chemical analysis (Horiguchi et al. 2000).

Gonad samples for histological examination were fixed in Bouin's fluid, embedded in paraffin and stained with Hematoxylin-Eosin. Scores for the development of reproductive cells were applied to quantitatively evaluate the gonadal maturation of abalone, using the developmental stages described in Tomita (1967, 1968). 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), which is the same as the method applied for *B. japonica*.

Chemical analysis of organotin (butyltin and phenyltin) compounds in tissues (muscle and/or gonad) of each abalone specimen was conducted by the method described in Horiguchi et al. (1994) with a certified reference material of Japanese sea bass, *Lateolabrax japonicus*, for TBT and TPT analysis (National Institute for Environmental Studies; NIES CRM no. 11) for quality assurance and quality control (Horiguchi et al. 2000).

The proportion of artificially released abalone in Jogashima was approximately 90% in this study, much higher than that from Tsushima (less than 5%). Morphological features of the gonad/digestive gland differed between specimens from the two sites, being either horn-shaped (Tsushima) or blunt (Jogashima) (Horiguchi et al. 2000).

Temporal variations in the reproductive developmental score of the populations also differed between the two sites: gonad maturation of females and males was synchronous in abalone from Tsushima, but not in abalone from Jogashima ($p < 0.05$; Figs. 8.8a, b). This may indicate differences in fertilization rates between abalone from Tsushima and Jogashima, because successful fertilization is considered to result from synchronous release of eggs and sperm into seawater. Ovarian maturation also seemed to be suppressed in females from Jogashima, compared to Tsushima (Fig. 8.8b) probably due to the presence of immature females in Jogashima throughout the spawning season. Testicular maturation seemed to be more frequently

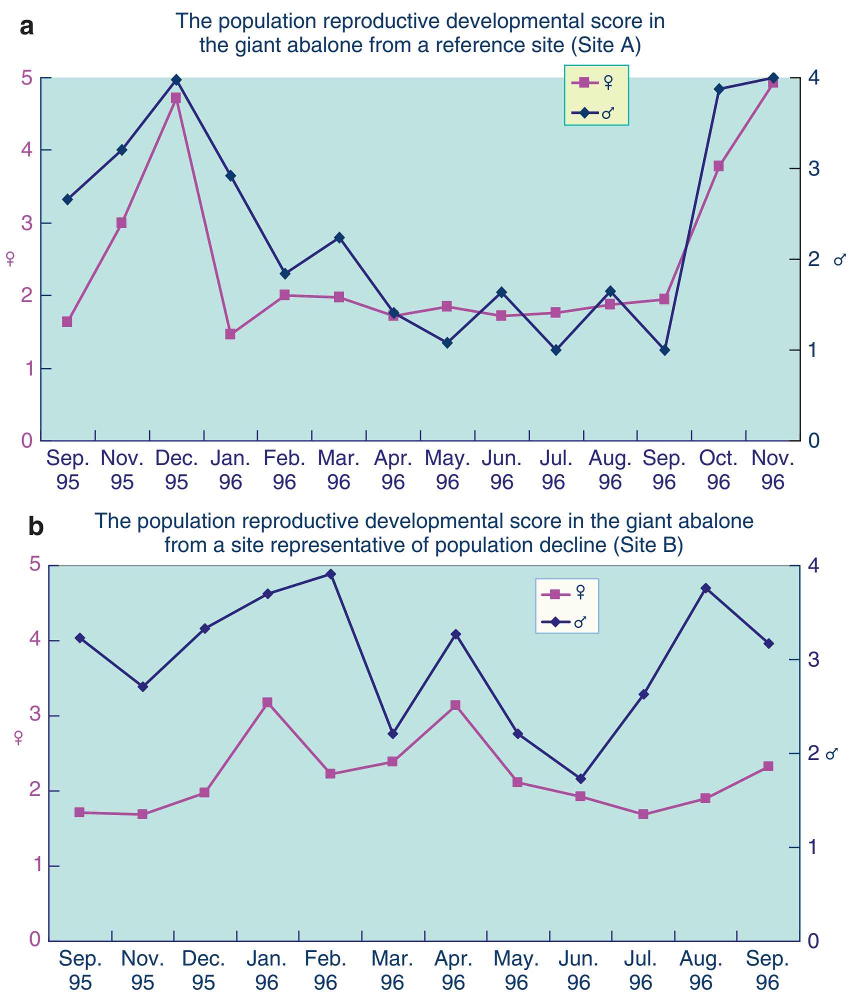

Fig. 8.8 Reproductive cycle of the giant abalone, *Haliotis madaka* (Horiguchi et al. 2000). (a) Tsushima, Nagasaki Prefecture, Japan (a reference site); (b) Jogashima, Kanagawa Prefecture, Japan (a site representative of areas where abalone populations have declined drastically)

observed in male abalone from Jogashima than from Tsushima (Fig. 8.8b). These gonadal features possibly suggest low reproductive success in giant abalone populations around Jogashima (Horiguchi et al. 2000).

Eleven of 54 females (approximately 20%) from Jogashima were observed to be masculinized; most of the gonadal tissues were ovaries with a small amount of testis tissue (i.e. an ovo-testis) (Horiguchi et al. 2000; Fig. 8.9). Either spermatogenesis (13%) or seminiferous tubule-like structure formation (8%) was observed (Horiguchi et al. 2000). This phenomenon of ovo-testis formation is basically similar to imposex in meso- and neogastropods, which is known to be induced

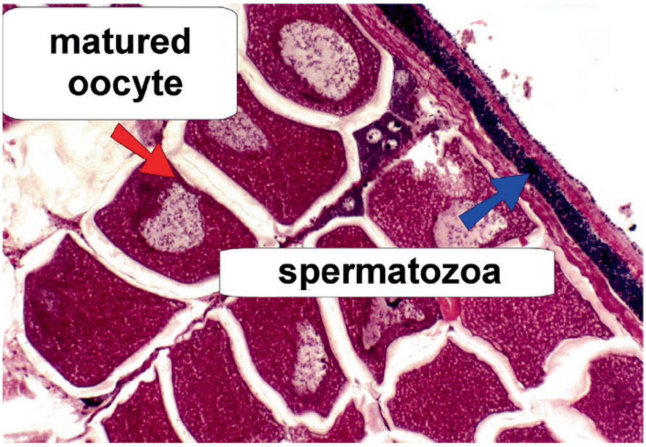

Fig. 8.9 Spermatogenesis in ovary of the giant abalone (*H. madaka*) (Horiguchi et al. 2000). This masculinized female abalone was collected at Jogashima, Japan in April 1996

by organotin compounds, such as TBT and TPT from antifouling paints, although no penis formation is observed in abalone (Smith 1971; Gibbs et al. 1987, 1988; Horiguchi et al. 1997a). More than 150 species of gastropods (neo- and mesogastropods) worldwide have been reported to be affected by imposex, as mentioned above. Intersex, i.e. the masculinization of female accessory sex organs was observed in the periwinkle *Littorina littorea,* reportedly caused by TBT (Bauer et al. 1995). Both imposex and intersex involve reproductive failure in severely affected individuals (Gibbs and Bryan 1986; Gibbs et al. 1988; Oehlmann et al. 1996). Thus, organotin compounds, such as TBT, may similarly affect the reproductive systems in archaeogastropods including abalone.

Concentrations of TBT and TPT in the muscle of abalone from Jogashima ($n = 83$) of 4.9 ± 4.4 and 6.3 ± 6.6 ng/g wet wt, respectively, were significantly higher than those from Tsushima ($n = 125$) ($p < 0.01$) of 0.8 ± 0.8 and 0.6 ± 1.3 ng/g wet wt, respectively. Organotin concentrations in ovary and testis were 10 to 20 times higher than those in muscle (e.g. average concentrations of TBT and TPT in the ovary of abalone from Tsushima were 31.8 and 22.1 ng/g wet wt, while those in the testis were 22.7 and 14.6 ng/g wet wt, respectively.). Therefore, it was suspected that organotin pollution had caused masculinization of female giant abalone from Jogashima (Horiguchi et al. 2000).

In addition, a 7-month *in situ* exposure experiment was conducted, using 40 abalone from Tsushima that were caged near a shipyard in Jogashima, from June 1998 to January 1999 (from the immature to the mature stage). The exposed abalone were fed brown algae, *Ecklonia cava*, once or twice a week during the experimental period. They were collected in January 1999 for histological examination and chemical analysis. This 7-month *in situ* exposure experiment resulted in spermatogenesis in the ovary of approximately 90% of exposed females (Horiguchi et al. 2000; Fig. 8.10). TBT and TPT levels in the muscle of the abalone were from

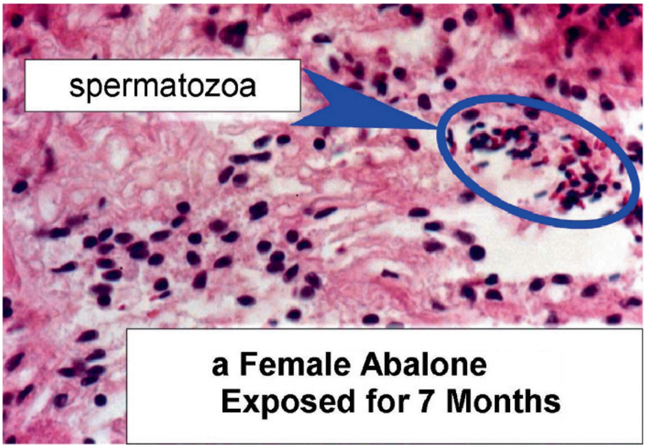

Fig. 8.10 Spermatogenesis in ovary of abalone (*H. gigantea*) exposed *in situ* near a shipyard in Jogashima, Japan (Horiguchi et al. 2000). Ovarian spermatogenesis was observed in approximately 90% of exposed females (collected from reference site, Tsushima, Japan and then used for this *in situ* exposure experiment for 7 months (from June 1998 to January 1999))

0.9 ± 0.4 and 1.3 ± 1.4 ng/g wet wt (n = 15), to 5.0 ± 0.2 and 21.5 ± 2.1 ng/g wet wt (n = 40), respectively ($p < 0.01$) (Horiguchi et al. 2000).

Subsequently, 2-month flow-through exposure experiments of TBT and TPT were conducted with abalone, *Haliotis gigantea,* to examine whether TBT and/or TPT induced spermatogenesis in females. Nominal concentrations of 100 ng/l of TBT and 100 ng/l of TPT caused significant formation of spermatids, spermatozoa and seminiferous tubule-like structures (spermatogenesis) in ovaries of exposed females (Horiguchi et al. 2002; Fig. 8.11). There were also significantly more contracted primary oocytes observed in ovaries of females exposed to either TBT or TPT than in ovaries of controls (Horiguchi et al. 2002). No significant histological changes were observed in testis of exposed males (Horiguchi et al. 2002). This ovarian spermatogenesis caused by TBT and/or TPT exposure seems very similar to the masculinization of mesogastropods and neogastropods, such as imposex. Remarkably high concentrations of TBT and TPT were observed in the head (including ganglia of the central nervous system), compared to concentrations in muscles: 68.32 ± 4.75 ng TBT/g and 1406.39 ± 11.32 ng TPT/g in the head, compared to 2.38 ± 0.81 ng TBT/g and 126.07 ± 68.04 ng TPT/g in muscles (on a wet tissue basis) (Horiguchi et al. 2002). Accumulation of TBT and TPT in the head may disturb reproductive hormonal regulators through neuropeptides released from ganglia. This may be one of the inducers for spermatogenesis in the ovaries of female abalone.

Thus, it was hypothesized that endocrine disruption, resulting in spermatogenesis in the ovary of giant abalone around the shipyard in Jogashima, was caused by TBT and/or TPT, and that organotin compounds from antifouling paints could be one of the causal factors of the observed abalone population decline.

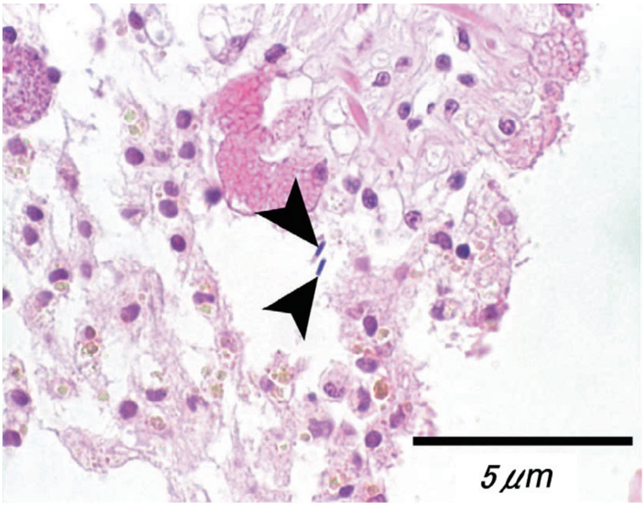

Fig. 8.11 Spermatogenesis in ovary of abalone (*H. gigantea*) exposed to 100 ng/l of TBT in a laboratory flow-through exposure system for 2 months. Ovarian spermatogenesis was observed in female *H. gigantea*, collected at a reference site, Tsushima, Japan and then used for this flow-through exposure experiment

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 (Horiguchi et al. 2005). 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 same as in Horiguchi et al. (2000), to evaluate the degree of sexual maturation by calculating the mean value of a histogram of these scores for the reproductive cells of each abalone (Horiguchi et al. 2005). The temporal variation in the degree of sexual maturation showed that female and male abalone from Tsushima matured synchronously, while those from Jogashima did not (Horiguchi et al. 2005), which was similar to results of the previous study during September 1995–November 1996 (Horiguchi et al. 2000). Approximately 19% of female abalone from Jogashima were masculinized with an ovo-testis (Horiguchi et al. 2005), which was also similar to the results of Horiguchi et al. (2000). Chemical analyses showed that concentrations of total butyltins (TBT, dibutyltin (DBT) and monobutyltin (MBT): ΣBTs) and total phenyltins (TPT, diphenyltin (DPT) and monophenyltin (MPT): ΣPTs) in the muscle of abalone from Jogashima ($n = 73$) of 7.8 ± 9.0 and 4.5 ± 6.8 ng/g wet wt, respectively, were significantly higher than those from Tsushima ($n = 87$) of 4.7 ± 4.9 and 0.8 ± 1.7 ng/g wet wt, respectively ($p < 0.05$ for ΣBTs; $p < 0.001$ for ΣPTs) (Horiguchi et al. 2005). Concentrations of TBT and TPT in the muscle of abalone from Jogashima ($n = 73$)

of 2.2 ± 2.5 and 5.8 ± 5.1 ng/g wet wt, respectively, were insignificantly and significantly higher than those from Tsushima (n = 87) of 0.4 ± 0.6 and 0.5 ± 0.9 ng/g wet wt, respectively ($p > 0.05$ for TBT; $p < 0.001$ for TPT) (Horiguchi et al. 2005). Thus, endocrine disruption as well as contamination by organotins in the giant abalone from Jogashima is still persisting.

8.5 Legislation Affecting Production, Import and Use of Organotin Compounds in Japan, and Future Perspectives on Organotin Pollution and Gastropod Populations

The causative agents of gastropod imposex and intersex, together with masculinization of female abalone, namely TBT and TPT compounds (TBTs and TPTs) have been used worldwide as antifouling agents in paints for ships and fishing nets since the mid-1960s, although TPT use has been much lower compared to TBT (Horiguchi et al. 1994; Goldberg 1986).

In Japan, 14 TBTs and 7 TPTs have been registered as existing chemical substances by the government (Horiguchi et al. 1994). Approximately 70%, 20% and 10% of total amount of TBTs produced and/or imported in Japan had been used in antifouling paints for ships, those for fishing nets and the other purposes (e.g., materials for different TBT species), respectively (Horiguchi et al. 1994). TPTs had also been used for agricultural chemicals (Horiguchi et al. 1994). Production, import and use of TBTs and TPTs have been strictly regulated in Japan since 1990 (Horiguchi et al. 1994). These uses of tri-organotins were reported to have been completely stopped by 1997, although evidence suggests illegal TBT use in antifouling paints in some areas (Horiguchi 2000; Horiguchi et al. 1994; Horiguchi et al., unpublished data, 2001).

A new international treaty, the International Convention on the Control of Harmful Anti-fouling System on Ships (abbreviated to AFS Convention) was adopted at the International Maritime Organization (IMO) in October 2001 for the worldwide ban of TBT and TPT (IMO 2001). The AFS Convention is expected to finally come into effect in September 2008, and field surveys should continue to be conducted to observe reproductive, anatomical, histopathological and ecological effects on affected molluscan populations together with temporal trend monitoring.

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