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Development of an *in vivo* acute bioassay using the marine medaka *Oryzias melastigma*

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Abstract

To determine whether the marine medaka *Oryzias melastigma* is a suitable model organism for *in vivo* acute toxicity bioassay in seawater, we first determined whether there were differences in the concentrations of chemicals that were toxic to marine medaka (*O. melastigma*) and freshwater medaka (*O. latipes*). We performed *in vivo* acute toxicity bioassay with 3-chloroaniline, triclosan, 3,4-dichloroaniline, fenitrothion, and pyriproxyfen on larvae of both species. Although the concentrations of 3-chloroaniline and fenitrothion that were lethal to the larvae were identical for both species, the toxic concentrations of triclosan, 3,4-dichloroaniline, and pyriproxyfen were lower for *O. melastigma* than for *O. latipes*. We then used an *in vivo* acute toxicity bioassay to monitor the quality of coastal seawater in Akita, Japan. No lethal effects were observed in the harbor and canal in 2019. *O. melastigma* could be used to monitor the quality of seawater with salinities in the range 2–25. Our findings suggest that *O. melastigma* can be used as the test fish for *in vivo* acute toxicity bioassay intended for water quality monitoring.

Keywords Aquatic organisms · Coastal water · Ecotoxicity · Marine pollution · Water toxicity

[redacted]

Declarations

Funding

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Competing Interests

The authors declare that they have no conflict of interest.

Availability of data and materials

The authors confirm that all data underlying the findings are fully available without restriction.

Authors contributions

All authors listed on the current study contributed to the experimental design or data analysis. (Yoshifumi Horie; All experiment except sampling for *in vivo* acute bioassays: Chiho Takahashi; sampling for *in vivo* acute bioassays).

Ethical approval

The fish which was used in the present study were handled according to guidelines of Akita Prefectural University.

Consent to participate

This research did not involve human subjects, so clinical trial registration is not applicable.

Consent for publish

The authors certify that this manuscript is our original unpublished work, has not been published elsewhere, and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission.

Introduction

In recent years, marine pollution has become a serious environmental problem. Examples include the plastic waste problem (reviewed by Campos da Rocha et al. 2021; Fauziah et al. 2021; Issac and Kandasubramanian 2021) and pollution by chemical substances used in the aquaculture industry (reviewed by Thuy et al. 2011; Zheng et al. 2021). To protect marine ecosystems, it is necessary to evaluate the effects of various marine pollutants on aquatic organisms.

Oryzias melastigma is a small fish native to India. Adult *O. melastigma* are about 5 cm in length, and their short generation time, which can be as brief as ~3 months, makes *O. melastigma* easy to breed. *O. melastigma* have therefore been popular models for the study of biological effects of various marine pollutants. For example, Wang et al. (2021) have reported that polystyrene microplastics depress hatching success, suppress body size and gonadosomatic index, and accelerate sexual maturity of *O. melastigma*. In addition, various chemicals, including acrylamide (Yue et al. 2021), nickel (Wang et al. 2020a), phenanthrene (Zheng et al. 2020), copper (Wang et al. 2020b), and difenoconazole (Dong et al. 2018) have been reported to have adverse effects on *O. melastigma*. We have recently conducted a comparative study of the toxicity of organotin compounds to freshwater Japanese medaka (*O. latipes*), which is a species closely related to *O. melastigma.* The study has revealed that the negative effects of exposure of *O. melastigma* and *O. latipes* to TPT or TBT follow identical trends; the lowest observed effect concentrations for survival and embryo development were the similar in both species (Horie et al. 2018; 2019). Although these previous studies have suggested that *Oryzias* congeners are useful small fish for assessment of the ecological risks of chemicals in freshwater and marine ecosystems, only the studies of Horie et al. (2018, 2019) have used the same chemicals and experimental methods to determine whether there are differences between the toxicities of chemicals to different *Oryzias* congeners.

Monitoring of water quality is an important step in assessing the risk of chemical pollution. Effect-based assessment using *in vivo* bioassays is one of the tools that has been applied in water quality monitoring (Escher et al. 2018). Effect-based methods have been applied to screen for adverse effects on fish in surface waters (Chen et al. 2015; Cristiano et al. 2020; Tamura et al. 2017; Zhang et al. 2015) or in wastewater (Leris et al. 2019; Maier et al. 2015; Wittlerová et al. 2020). To our knowledge, however, no previous study has adapted *in vivo* bioassays using *O. melastigma* to monitor seawater quality, although Yamagishi et al. (2018) have used *in vivo* bioassays with the marine cyanobacterium *Cyanobium* sp. NIES-981 to evaluate the toxicity of leaches from hydrothermal sulfide deposits.

In this study, we first compared the toxicities of various chemical substances including 3-chloroaniline, triclosan, 3,4-dichloroaniline, Fenitrothion, and pyriproxyfen between *O. melastigma* and *O. latipes*. We then used *in vivo* acute toxicity bioassay with *O. melastigma* to monitor coastal water quality in Akita Prefecture, Japan.

Materials and methods

Test fish

The National Institute for Environmental Studies, Tsukuba, Japan, supplied the NIES-R strain of *O. latipes*, which has been maintained since 2017 under an artificial photoperiod of 16-h/8-h light/dark at 25 ± 2 °C at Akita Prefectural University.

The *O. melastigma*, which is derived from individuals originally purchased from a local pet shop, has been maintained since 2017 under an artificial photoperiod of 16-h/8-h light/dark at 25 ± 2 °C and a salinity of 17 ± 2 at Akita Prefectural University. The identification of the species was confirmed by using 12S and 16S ribosomal RNA genes (Takehana et al. 2005). Artificial seawater was prepared from seawater salts (Marine ART Hi, Osaka Yakken Co. Ltd, Osaka, Japan). In all experiments, the medaka were handled in a humane manner in accordance with the guidelines of Akita Prefectural University, Japan.

Test chemicals and exposure concentration

The chemicals 3-chloroaniline (CAS no. 108-42-9; purity, >99%), triclosan (3380-34-5; >98%), and 3,4-dichloroaniline (95-76-1; >98%) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fenitrothion (122-14-5; >98%) and pyriproxyfen (95737-68-1; >99%) were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). We selected these test chemicals because we already reported the lethal toxicity of these chemical substances using zebrafish *Danio rerio* in previous reports (Horie et al., 2017).

We used exposure concentrations of 0 (control), 6, 12, 25, or 50 mg/L 3-chloroaniline, 0 (control), 150, 300, 600, or 1200 μg/L triclosan, 0 (control), 31, 62, 125, or 250 μg/L 3,4-dichloroaniline, 0 (control), 1.75, 3.5, 7, or 14 mg/L fenitrothion, and 0 (control), 0.3, 0.6, 1.2, or 2.5 mg/L pyriproxyfen.

In vivo acute toxicity bioassay using *O. melastigma* and *O. latipes*

A 500-mL glass beaker (exposure volume of 400 mL) and larvae within 5 days after hatching were used for the acute toxicity tests. Ten larvae were distributed in each glass beaker, and four replicate 500-mL glass beakers were used for each concentration. A total of 40 larvae were therefore used for each treatment. The test period was 96 hours, and observations for dead larvae were performed every 24 hours. Dead larvae were removed and the exposure test water was changed every day. Acute toxicity tests with *O. melastigma* and *O. latipes* were conducted at 25 ± 2 °C and a photoperiod of 16 h light:8 h dark. The salinity was 17 ± 2 in the experiments with *O. melastigma*. The survival rate was calculated after each test had been completed.

Study area and sampling for *in vivo* acute bioassays

The targets of this study were the harbor and canal of the port of Akita. This port, the largest in the Pan-Japan Sea area, is located in the western part of Akita City, in the northeastern part of Akita Prefecture (Fig. 1). Site A was in a coastal area where wastewater from a thermal power plant is discharged. Site B was located near a paper mill factory.

Seawater samples were collected from each site in May, August, and October of 2019 and February of 2020. Surface seawater samples were taken from a depth of 0.3 m below the surface. Twenty liters of seawater were sampled from each site for *in vivo* acute bioassays using *O. melastigma*. Seawater samples were transported to the laboratory and maintained at 4 ℃ until the *in vivo* acute bioassays. Characteristics of the seawater were determined with a combination pH and electrical conductivity meter (WQ-310; Horiba, Kyoto, Japan), dissolved oxygen meter (OM-71; Horiba, Kyoto, Japan), salinity meter (YK-31SA; Mother tool, Nagano, Japan), and a thermometer (AD-5624; AND, Tokyo, Japan).

In vivo acute toxicity bioassay using *O. melastigma*

The seawater sample, which had been stored at 4 ° C, was heated to 25 °C using a water bath. Next, one treatment consisting of the seawater sample and four additional treatments consisting of a control treatment (artificial seawater) and treatments corresponding to 12.5%, 25%, and 50% of the sampled seawater were prepared using artificial seawater. The salinity of the artificial seawater was identical to the salinity of the seawater sample. The experiments were then carried out in the same way as the acute toxicity tests using *O. melastigma.*

Statistical analyses

All data were analyzed using Excel software (Microsoft, Redmond, WA, USA), R software ver 3.5.1, and the R package " Rcmdr " (Fox and Bouchet-Valat, 2018). Statistical analyses were conducted as follows: (1) Bartlett's test was used to test for the equality of k variances (significance level, 5%). (2) If the null hypothesis that the variances of the k sampled populations were equal was confirmed (i.e., the data were homoscedastic) ($p > 0.05$ based on Bartlett's test), Dunnett's multiple comparison test was performed to test for differences in mean values. (3) If the null hypothesis that the variances of the k sampled populations were equal was rejected (i.e., the data were heteroscedastic) ($p < 0.05$ based on Bartlett's test), Steel's test was used. We calculated the lowest-observed-effect concentration (LOEC) for each endpoint according to the Organization for Economic Cooperation and Development Test Guideline 210 (OECD, 2013). The LOEC is the lowest test concentration at which the substance is observed to have a statistically significant effect.

Results

Comparison of the toxicity of chemical substances between *O. melastigma* and *O. latipes*

Figure 2 shows the survival rates following exposure to each chemical. We observed no mortality in the control group of either species, and survival rates decreased in a concentration-dependent manner in all exposures. In the 3-chloroaniline exposure, a significant decrease in survival rate compared to the control group was observed in the 25 and 50 mg/L concentration groups of both species (Fig. 2a, b). In the triclosan exposure, all larvae of both species died at exposures of 600 and 1200 µg/L (Fig. 2c, d). In the 3,4-dichloroaniline treatments, all exposures caused a significant decrease of the survival of *O. melastigma* (Fig. 2e). All *O. latipes* larvae exposed to 125 or 250 µg/L of 3,4-dichloroaniline died, and there was a significant decrease of their survival when larvae were exposed to 62 µg/L of 3,4-dichloroaniline (Fig. 2f). All larvae of *O. melastigma* died when exposed to 3.5, 7, or 14 mg/L of fenitrothion (Fig. 2g). All larvae of *O. latipes* died when exposed to 7 or 14 mg/L of fenitrothion, and the survival rate decreased significantly when larvae were exposed to 3.5 mg/L of fenitrothion (Fig. 2h). All larvae of both species died when exposed to 1.2 or 2.5 mg/L of pyriproxyfen (Fig. 2i, j).

Table 1 compares the lethal LOECs of *O. melastigma* and *O. latipes*. The LOECs of 3-chloroaniline and fenitrothion were similar in the two species. However, the LOECs of triclosan, 3,4-dichloroaniline, and pyriproxyfen were lower in *O. melastigma* than in *O. latipes*.

Monitoring water quality in Akita harbor and canal

Table 2 shows the values of the physiochemical variables monitored in water samples from Akita harbor and canal. The water temperatures at both sites were lowest in February (Site A, 9.7 °C; Site B, 8.7 °C) and highest in August (Site A, 27.6 °C; Site B, 25.8 °C). The pH values were stable throughout the monitoring period and fell in the range 7.1–8.21. Salinity differed between site A and site B. The salinity at site A was stable throughout the year at 24–25. The salinity at site B was lower and varied between 2 and 8. The conductivity of the water was highest at site A in October (39.8 µS/cm). The lowest conductivities were recorded in August, and the minimum conductivity was 6.6 µS/cm at Site B. The dissolved oxygen concentrations were stable throughout the monitoring period at both sites and fell in the range 4.51–6.23 mg/L.

Figures 3 and 4 show the larval survival rates following exposure to water from Site A and Site B, respectively. Bioassays were performed a total of four times at each site, in spring (May), summer (August), autumn (October), and winter (February). The survival rates were high (80% or more) at all exposure concentration in Site A and Site B throughout the year; no significant adverse effect on survival was observed compared to the control (artificial seawater).

Discussion

In the present study, we determined the concentrations of 3-chloroaniline, triclosan, 3,4-dichloroaniline, fenitrothion, and pyriproxyfen that were acutely toxic to *O. melastigma* and *O. latipes*. Although there have been no reports of 3-chloroaniline and 3,4-dichloroaniline in samples of water from natural systems, the other chemicals have been detected in both freshwater and seawater. Triclosan has been detected at 10 ng/L from the Ruhr River in Germany (Bester 2005), at 90.2–478 ng/L in the Shijing River, China

(Zhao et al. 2010), at 11–31 ng/L in the Tone Canal, Japan (Nishi et al. 2008), and at 0.55–10.5 ng/L in marine waters near Singapore (Bayen et al. 2013). Fenitrothion has been detected at 680.6 ng/L in the Ebro River, Spain (Kuster et al. 2008), and at 370.0 ng/L in the Kurose River, Japan (Kaonga et al. 2015). Pyriproxyfen has been detected at 82.92–99.59 ng/L in the Júcar River, Spain (Belenguer et al. 2014), at up to 950 ng/L in the Nile River, Egypt (Ghani and Hanafi 2016), and at the detection limit of 10 ng/L in the coastal waters of Japan (Añasco et al. 2010). To determine the biological risk associated with a chemical, it is necessary to know the concentration in the aquatic environment and the LOEC of the chemical. The LOECs determined in the present study (Table 1) were all far higher than their environmental concentrations.

We showed that the LOEC for death differed between *O. melastigma* and *O. latipes*. To date, few studies have compared the toxicity of chemicals to both freshwater and saltwater species of fish of the same genus. Bosker et al. (2017) have reviewed the effects of endocrine-disrupting chemicals on the reproduction of species of *Oryzias*. The lowest concentrations of 17α-ethinylestradiol that have been observed to exert an adverse effect on the fecundity of a species of *Oryzias* differ by a factor of 10 between species: 50 ng/L for *O. melastigma* (Lee et al. 2014) and 500 ng/L for *O. latipes* (Seki et al. 2002). In addition, the LOECs of bisphenol A with respect to the fecundity of species of *Oryzias* differ by a factor of 20: 50 μg/L for *O. melastigma* (Huang et al. 2018) and 1000 μg/L for *O. latipes* (Horie et al., unpublished data). However, the acutely toxic LC50 values of copper are similar for *O. melastigma*, 1300 μg/L (Yi et al. 2017), and for *O. latipes*, 1100 μg/L (Tsuji et al. 1986). In addition, our research group has recently reported that the concentrations of tributyl tin as well as triphenyl tin that are lethal to *O. melastigma* and *O. latipes* are identical (Horie et al. 2019). In the present study, we found that the LOECs of 3-chloroaniline and fenitrothion were identical for both species. The LOECs of triclosan, 3,4-dichloroaniline, and pyriproxyfen were lower for *O. melastigma* than for *O. latipes*, although lethal effects are very consistent. These previous reports may suggest that when assessing the risk that a chemical poses to marine fish, the risk cannot be predicted from the concentration that is toxic to freshwater fish.

Bioassays can be used to comprehensively evaluate the toxicity of water by exposing aquatic organisms to the water and determining the presence or absence of biological effects. For example, many studies have evaluated the degree of pollution of natural waters by *in vivo* bioassays using fish such as zebrafish (Tiber River; Cristiano et al. 2020: Panamanian rivers; Wilson et al. 2021), Murray rainbowfish (Murray-Darling River; Vajda et al. 2015), and rainbow trout (Argen River; Maier et al. 2015).

However, no previous study has adapted *in vivo* bioassays using marine fish to monitor the quality of seawater. The salinity at each sampling point in a coastal area can differ (NASA Salinity, https://salinity.oceansciences.org/). Furthermore, in this study there were temporal changes of the salinity at the same sampling point. The implication is that assessments of coastal water quality via *in vivo* bioassays using marine fish must be done with a euryhaline species of fish. *O. melastigma* is highly tolerant to changes of salinity and readily acclimates to freshwater and seawater environments (Inoue and Takei 2002; Horie et al. 2019). The work reported here was the first study to monitor the quality of seawater using *O. melastigma* in Akita harbor and canal, within which the range of salinity is 2–25. In the future, it will be necessary to carry out water quality monitoring using *in vivo* bioassays in a variety of coastal waters to clarify the effectiveness of bioassays.

Conclusions

To develop an *in vivo* acute bioassay using marine medaka, we first examined the differences between the concentrations of several chemicals that were toxic to marine medaka (*O. melastigma*) versus freshwater medaka (*O. latipes*). The bioassay must then be performed using natural seawater over a relevant range of salinities. This study was the first to use *in vivo* acute bioassays with *O. melastigma* as a tool to monitor the quality of seawater. The discovery that the toxicity of triclosan, 3,4-dichloroaniline, and pyriproxyfen differed between marine and freshwater species of medaka underlines the importance of using marine organisms to evaluate the ecological effects of chemicals in the ocean. The fact that *O. melastigma* can be used to monitor the quality of seawater in harbors and canals with salinities in the range 2–25 suggests that *O. melastigma* is a good model marine organism for *in vivo* fish bioassays used to monitor water quality.

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References

Añasco, N.C., Koyama, J., Uno, S. (2010). Pesticide residues in coastal waters affected by rice paddy effluents temporarily stored in a wastewater reservoir in southern Japan. *Archives of Environmental Contamination and Toxicology*, 58(2), 352-60. doi: 10.1007/s00244-009-9364-1.

Bayen, S., Zhang, H., Desai, M.M., Ooi, S.K., Kelly, B.C. (2013). Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: influence of hydrodynamics and physical-chemical properties. *Environmental Pollution*, 182,1-8. doi: 10.1016/j.envpol.2013.06.028.

Belenguer, V., Martinez-Capel, F., Masiá, A., Picó, Y. (2014). Patterns of presence and concentration of pesticides in fish and waters of the Júcar River (Eastern Spain). *Journal of Hazardous Materials,* 265, 271-279. doi: 10.1016/j.jhazmat.2013.11.016.

Bester, K. (2005). Fate of triclosan and triclosan-methyl in sewage treatment plants and surface waters. *Archives of Environmental Contamination and Toxicology*, 49(1), 9-17. doi: 10.1007/s00244-004-0155-4.

Bosker, T., Santoro, G., Melvin, S.D. (2017). Salinity and sensitivity to endocrine disrupting chemicals: A comparison of reproductive endpoints in small-bodied fish exposed under different salinities. *Chemosphere*, 183, 186-196. doi: 10.1016/j.chemosphere.2017.05.063.

Campos, da Rocha, F.O., Martinez, S.T., Campos, V.P., da Rocha, G.O., de Andrade, J.B. (2021). Microplastic pollution in Southern Atlantic marine waters: Review of current trends, sources, and perspectives. *Science of the Total Environment,* 782, 146541. doi: 10.1016/j.scitotenv.2021.146541.

Chen, T.H., Chen, Y.L., Chen, C.Y., Liu, P.J., Cheng, J.O., Ko, F.C. (2015). Assessment of ichthyotoxicity and anthropogenic contamination in the surface waters of Kenting National Park, Taiwan. *Environmental Monitoring and Assessment,* 187(5), 265. doi: 10.1007/s10661-015-4511-9.

Cristiano, W., Lacchetti, I., Di Domenico, K., Corti, M., Mancini, L., Carere, M. (2020). Application of effect-based methods (EBMs) in a river basin: a preliminary study in Central Italy. *Annali dell'Istituto Superiore di Sanità,* 56(1), 114-121. doi: 10.4415/ANN_20_01_16.

Dong, X., Zhang, L., Chen, M., Yang, Z., Zuo, Z., Wang, C. (2018). Exposure to difenoconazole inhibits reproductive ability in male marine medaka (Oryzias melastigma). *Journal of Environmental Sciences,* 63, 126-132. doi: 10.1016/j.jes.2017.05.030.

Escher, B.I., Aït-Aïssa, S., Behnisch, P.A., Brack, W., Brion, F., Brouwer, A., Buchinger, S., Crawford, S.E., Du Pasquier, D., Hamers, T., Hettwer, K., Hilscherová, K., Hollert, H., Kase, R., Kienle, C., Tindall, A.J., Tuerk, J., van der Oost, R., Vermeirssen, E., Neale, P.A. (2018). Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Science of the Total Environment,* 628-629, 748-765. doi: 10.1016/j.scitotenv.2018.01.340.

Fauziah, S.H., Rizman-Idid, M., Cheah, W., Loh, K.H., Sharma, S. M. R. N., Bordt, M., Praphotjanaporn, T., Samah, A.A., Sabaruddin, J.S.B., George, M. (2021). Marine debris in Malaysia: A review on the pollution intensity and mitigating measures. *Marine Pollution Bulletin,* 167 , 112258. doi: 10.1016/j.marpolbul.2021.112258.

Fox, J., Bouchet-Valat, M. (2018). Rcmdr: R Commander. R package version 2.5-1.

Ghani, S.A., Hanafi, A.H. (2016). QuEChERS Method Combined with GC–MS for Pesticide Residues Determination in Water. *Journal of Analytical Chemistry*, 71(5), 508–512.

Horie, Y., Yamagishi, T., Takahasgi, H., Shintaku, Y., Iguchi, T., Tatarazako, N. (2017). Assessment of the lethal and sublethal effects of 20 environmental chemicals in zebrafish embryos and larvae by using OECD TG 212. *Journal of applied toxicology*, 37, 1245–1253.

Horie, Y., Kanazawa, N., Yamagishi, T., Yonekura, K., Tatarazako, N. (2018). Ecotoxicological test assay using OECD TG 212 in marine Java medaka (*Oryzias javanicus*) and freshwater Japanese medaka (*Oryzias latipes*). *Bulletin of Environmental Contamination and Toxicology*, 101, 344–348.

Horie, Y., Kanazawa, N., Suzuki, A., Yonekura, K., Chiba, T. (2019). Influences of Salinity and Organic Compounds on Embryo Development in Three Medaka Oryzias Congeners with Habitats Ranging from Freshwater to Marine. *Bulletin of Environmental Contamination and Toxicology*, 103(3), 411-415. doi: 10.1007/s00128-019-02649-3.

Huang, Q., Liu, Y., Chen, Y., Fang, C., Chi, Y., Zhu, H., Lin, Y., Ye, G., Dong, S. (2018). New insights into the metabolism and toxicity of bisphenol A on marine fish under long-term exposure. *Environmental Pollution,* 242(Pt A), 914-921. doi: 10.1016/j.envpol.2018.07.048.

Inoue, K., Takei, Y. (2002). Diverse adaptability in *Oryzias* species to high environmental salinity. *Zoological Science,* 19(7), 727–734.

Issac, M.N., Kandasubramanian, B. (2021). Effect of microplastics in water and aquatic systems. *Environmental Science and Pollution Research,* 2,1-19. doi: 10.1007/s11356-021-13184-2.

Kaonga, C.C., Takeda, K., Sakugawa, H. (2015). Diuron, Irgarol 1051 and Fenitrothion contamination for a river passing through an agricultural and urban area in Higashi Hiroshima City, Japan. *Science of the Total Environment*, 518-519,450-458. doi: 10.1016/j.scitotenv.2015.03.022.

Kuster, M., López de, Alda, M.J., Barata, C., Raldúa, D., Barceló, D. (2008). Analysis of 17 polar to semi-polar pesticides in the Ebro river delta during the main growing season of rice by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. *Talanta*, 75(2), 390-401. doi: 10.1016/j.talanta.2007.11.027.

Lee, P.Y., Lin, C.Y., Chen, T.H. (2014). Environmentally relevant exposure of 17α-ethinylestradiol impairs spawning and reproductive behavior in the brackish medaka *Oryzias melastigma*. *Marine Pollution Bulletin*, 85, 338–343.

Leris, I., Kalogianni, E., Tsangaris, C., Smeti, E., Laschou, S., Anastasopoulou, E., Vardakas, L., Kapakos, Y., Skoulikidis, N.T. (2019). Acute and sub-chronic toxicity bioassays of Olive Mill Wastewater on the Eastern mosquitofish Gambusia holbrooki. *Ecotoxicology and Environmental Safety,* 175, 48-57. doi: 10.1016/j.ecoenv.2019.03.025.

Maier, D., Blaha, L., Giesy, J.P., Henneberg, A., Köhler, H.R., Kuch, B., Osterauer, R., Peschke, K., Richter, D., Scheurer, M., Triebskorn, R. (2015). Biological plausibility as a tool to associate analytical data for micropollutants and effect potentials in wastewater, surface water, and sediments with effects in fishes. *Water Research,* 72,127-144. doi: 10.1016/j.watres.2014.08.050.

Nishi, I., Kawakami, T., Onodera, S. (2008). Monitoring of triclosan in the surface water of the Tone Canal, Japan. *Bulletin of Environmental Contamination and Toxicology*, 80(2),163-166. doi: 10.1007/s00128-007-9338-9.

OECD. 2013. Guidelines for the Testing of Chemicals, Test No. 210: Fish, Early-life Stage Toxicity Test. OECD Publishing.

Seki, M., Yokota, H., Matsubara, H., Tsuruda, Y., Maeda, M., Tadokoro, H., Kobayashi, K. (2002). Effect of ethinylestradiol on the reproduction and induction of vitellogenin and testis-ova in medaka (Oryzias latipes). *Environmental Toxicology and Chemistry,* 21(8),1692-1698.

Takehana, Y., Naruse, K., Sakaizumi, M. (2005). Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution,* 36(2): 417–428.

Tamura, I., Yasuda, Y., Kagota, K.I., Yoneda, S., Nakada, N., Kumar, V., Kameda, Y., Kimura, K., Tatarazako, N., Yamamoto, H. (2017). Contribution of pharmaceuticals and personal care products (PPCPs) to whole toxicity of water samples collected in effluent-dominated urban streams. *Ecotoxicology and Environmental Safety,* 144, 338-350. doi: 10.1016/j.ecoenv.2017.06.032.

Thuy, H.T., Nga le, P., Loan, T.T. (2011). Antibiotic contaminants in coastal wetlands from Vietnamese shrimp farming. *Environmental Science and Pollution Research,* 18(6), 835-841. doi: 10.1007/s11356-011-0475-7.

Tsuji, S., Tonogai, Y., Ito, Y., Kanoh, S. (1986). The influence of rearing temperature on the toxicity of various environmental pollutants for killfish (Orizas latipes). *EISEI KAGAKU*, 32(1):46-53. (Japanese)

Vajda, A.M., Kumar, A., Woods, M., Williams, M., Doan, H., Tolsher, P., Kookana, R.S., Barber, L.B. (2015). Integrated assessment of wastewater treatment plant effluent estrogenicity in the Upper Murray River, Australia, using the native Murray rainbowfish (Melanotaenia fluviatilis). *Environmental Toxicology and Chemistry,* 34(5), 1078-1087. doi: 10.1002/etc.2895.

Wang, Z., Yeung, K.W.Y., Zhou, G.J., Yung, M.M.N., Schlekat, C.E., Garman, E.R., Gissi, F., Stauber, J.L., Middleton, E.T., Lin Wang, Y.Y., Leung, K.M.Y. (2020a). Acute and chronic toxicity of nickel on freshwater and marine tropical aquatic organisms. *Ecotoxicology and Environmental Safety,* 206:111373. doi: 10.1016/j.ecoenv.2020.111373.

Wang, R.F., Zhu, L.M., Zhang, J., An, X.P., Yang, Y.P., Song, M., Zhang, L. (2020b). Developmental toxicity of copper in marine medaka (Oryzias melastigma) embryos and larvae. *Chemosphere*, 247, 125923. doi: 10.1016/j.chemosphere.2020.125923.

Wang, J., Zheng, M., Lu, L., Li, X., Zhang, Z., Ru, S. (2021). Adaptation of life-history traits and trade-offs in marine medaka (*Oryzias melastigma*) after whole life-cycle exposure to polystyrene microplastics. *Journal of Hazardous Materials,* 414,125537. doi: 10.1016/j.jhazmat.2021.125537.

Wilson, E.W., Castro, V., Chaves, R., Espinosa, M., Rodil, R., Quintana, J.B., Vieira, M.N., Santos, M.M. (2021). Using zebrafish embryo bioassays combined with high-resolution mass spectrometry screening to assess ecotoxicological water bodies quality status: A case study in Panama rivers. *Chemosphere*, 272, 129823. doi: 10.1016/j.chemosphere.2021.129823.

Wittlerová, M., Jírová, G., Vlková, A., Kejlová, K., Malý, M., Heinonen, T., Wittlingerová, Z., Zimová, M. (2020). Sensitivity of zebrafish (Danio rerio) embryos to hospital effluent compared to Daphnia magna and Aliivibrio fischeri. *Physiological Research,* 31, 69(Suppl 4):S681-S691.

Yamagishi, T., Fuchida, S., Katsumata, M., Horie, Y., Mori, F., Kitayama, A., Kawachi, M., Koshikawa, H., Nozaki, T., Kumagai, H., Ishibashi, J.I., Tatarazako, N. (2018). Evaluation of the toxicity of leaches from hydrothermal sulfide deposits by means of a delayed fluorescence-based bioassay with the marine cyanobacterium Cyanobium sp. NIES-981. *Ecotoxicology*, 27(10), 1303-1309. doi: 10.1007/s10646-018-1989-2.

Yi, X., Bao, V.W.W., Leung, K.M.Y. (2017). Binary mixture toxicities of triphenyltin with tributyltin or copper to five marine organisms: Implications on environmental risk assessment. *Marine Pollution Bulletin,* 124(2):839-846. doi: 10.1016/j.marpolbul.2017.02.031.

Yue, Z., Tian, E., Chen, Y., Luo, L., Yang, L., He, L., Li, L., Wang, J. (2021). The adverse effects of acrylamide exposure on the early development of marine medaka (Oryzias melastigma) and its mechanisms. *Marine Pollution Bulletin,* 163:111875. doi: 10.1016/j.marpolbul.2020.111875.

Zhao, J.L., Ying, G.G., Liu, Y.S., Chen, F., Yang, J.F., Wang, L. (2010). Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: from source to the receiving environment. *Journal of Hazardous Materials,* 179(1-3),215-222. doi: 10.1016/j.jhazmat.2010.02.082.

Zhang, L., Li, Q., Chen, L., Zhang, A., He, J., Wen, Z., Wu, L. (2015). Toxicity of surface water from Huangpu River to luminous bacteria (Vibrio qinghaiensis SP. Q67) and zebrafish (Danio rerio) embryos. *Ecotoxicology and Environmental Safety,* 112,137-143. doi: 10.1016/j.ecoenv.2014.10.037.

Zheng, D., Yin, G., Liu, M., Chen, C., Jiang, Y., Hou, L., Zheng, Y. (2021). A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. *Science of the Total Environment*, 777,146009. doi: 10.1016/j.scitotenv.2021.146009.

Zheng, Y., Li, Y., Yue, Z., Samreen, Li, Z., Li, X., Wang, J. (2020). Teratogenic effects of environmentally relevant concentrations of phenanthrene on the early development of marine medaka (Oryzia melastigma). *Chemosphere*, 254,126900. doi: 10.1016/j.chemosphere.2020.126900.

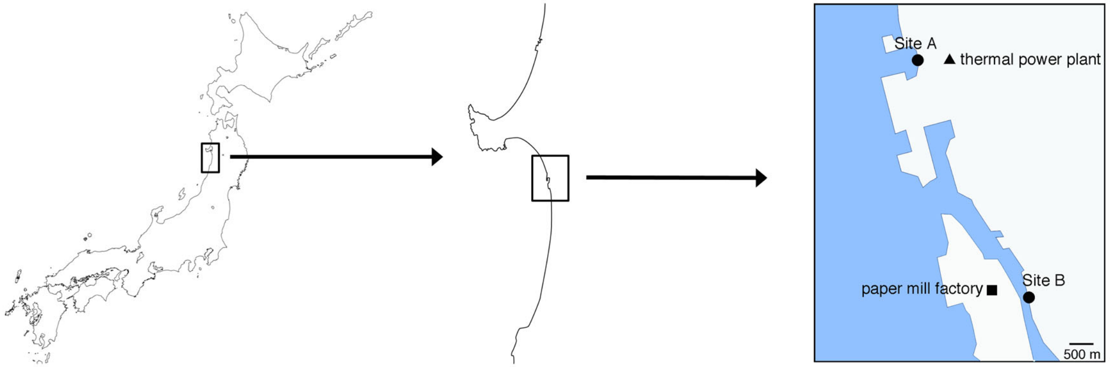
Figures

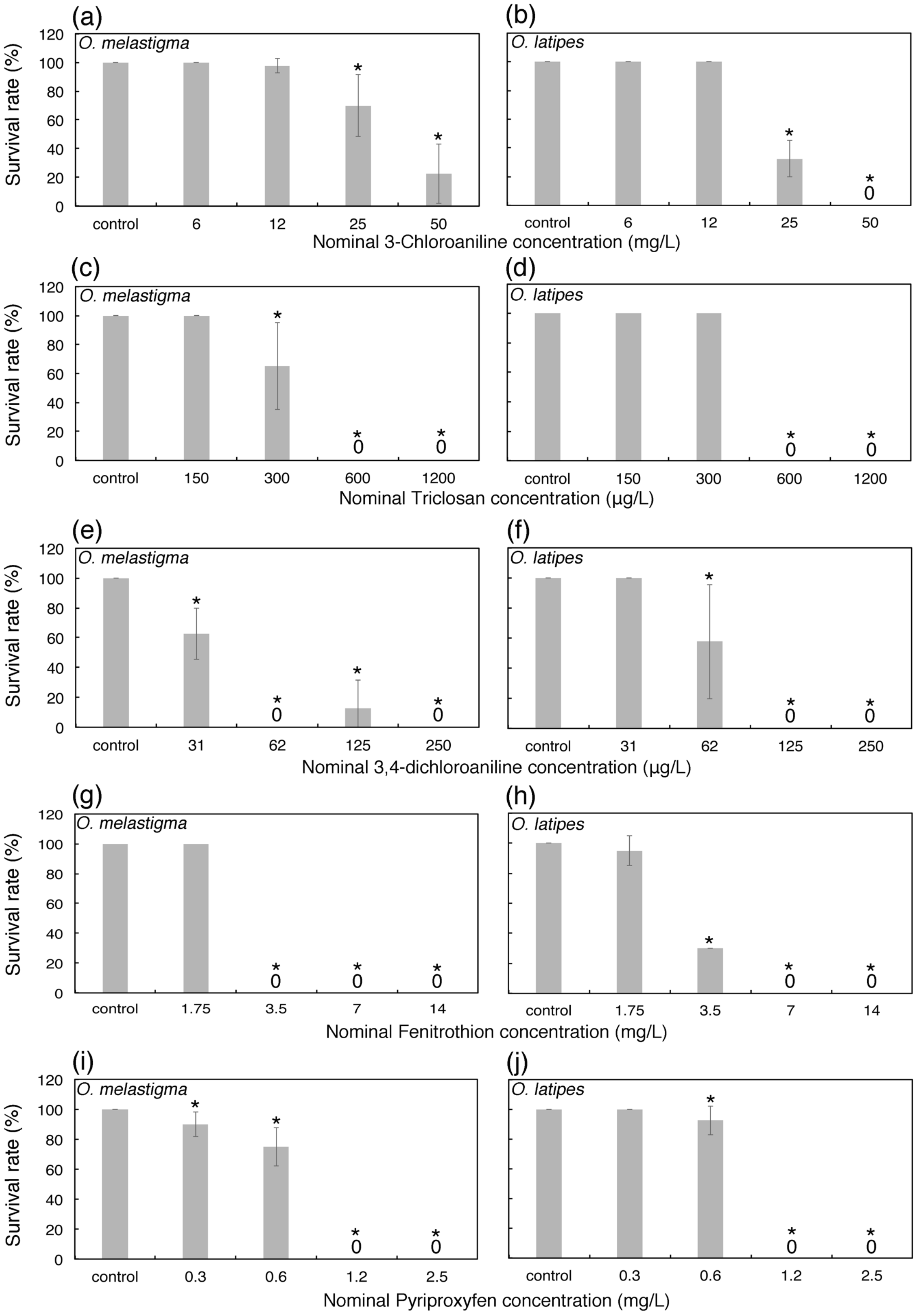
Fig. 1 Sampling locations in coastal waters of Akita, Japan. Samples were taken at sites A and B. Site A was in the harbor, and Site B was in the Akita canal

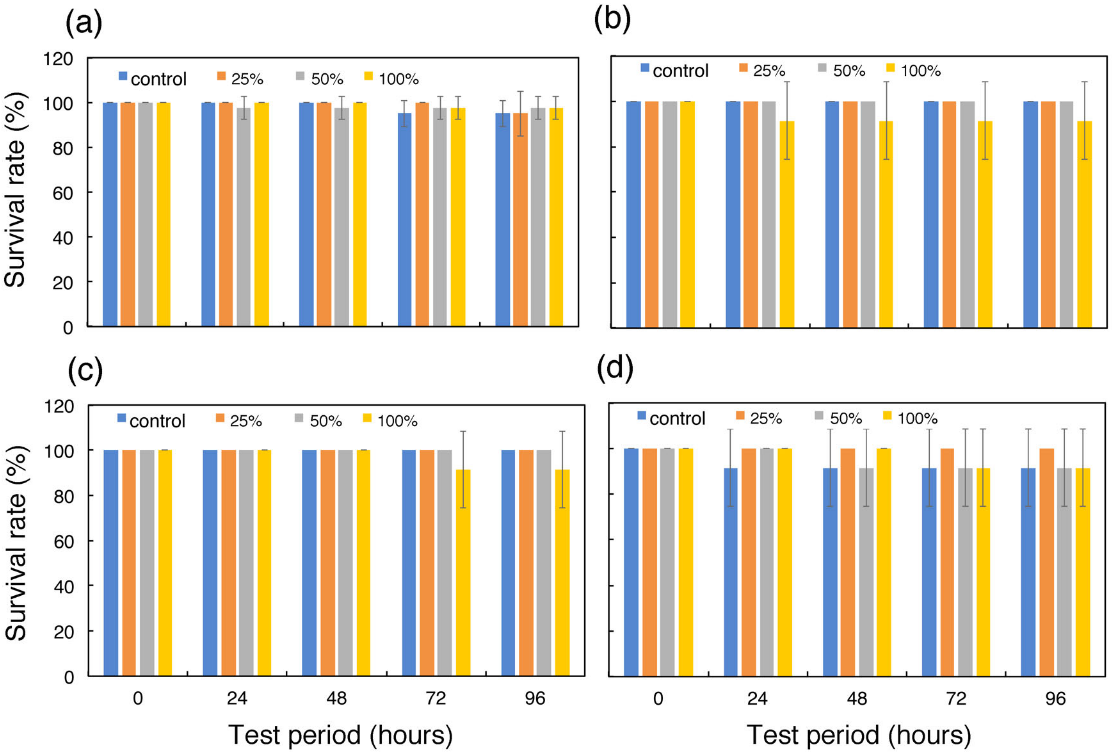
Fig. 2 Survival rates of *O. melastigma* (marine fish) and *O. latipes* (freshwater fish) larvae after exposure to the 5 test chemicals. Columns and error bars are means ± standard errors of the means ($n = 4$ per group). Asterisks indicate statistically significant differences compared with control (Dunnett's test or Steel's test; $P < 0.05$). (a, b) 3-chloroaniline, (c, d) triclosan, (e, f) 3,4-dichloroaniline, (g, h) fenitrothion, (i, j) pyriproxyfen. (a, c, e, g, i) *O. melastigma* and (b, d, f, h, j) *O. latipes*

Fig. 3 Results of *O. melastigma* acute toxicity test using Site A harbor water. Columns and error bars are means ± standard errors of the mean ($n = 4$ per group). Errors are zero for 100% survival. (a) May, (b) August, (c) October, (d) February

Fig. 4 Results of *O. melastigma* acute toxicity tests using Site B Akita canal water. Columns and error bars are means ± standard errors of the mean ($n = 4$ per group). Errors are zero for 100% survival. (a) May, (b) August, (c) October, (d) February


Site A
thermal power plant
paper mill factory
Site B
500 m


(a)
O. melastigma
(b)
O. latipes
Survival rate (%)
120
100
80
60
40
20
0
control
6
12
25
50
*
0
Nominal 3-Chloroaniline concentration (mg/L)
(c)
O. melastigma
(d)
O. latipes
control
150
300
600
1200
Nominal Triclosan concentration (μg/L)
(e)
O. melastigma
(f)
O. latipes
control
31
62
125
250
Nominal 3,4-dichloroaniline concentration (μg/L)
(g)
O. melastigma
(h)
O. latipes
control
1.75
3.5
7
14
Nominal Fenitrothion concentration (mg/L)
(i)
O. melastigma
(j)
O. latipes
control
0.3
0.6
1.2
2.5
Nominal Pyriproxyfen concentration (mg/L)


(a)
(b)
(c)
(d)
control
25%
50%
100%
Survival rate (%)
0
20
40
60
80
100
120
0
24
48
72
96
Test period (hours)

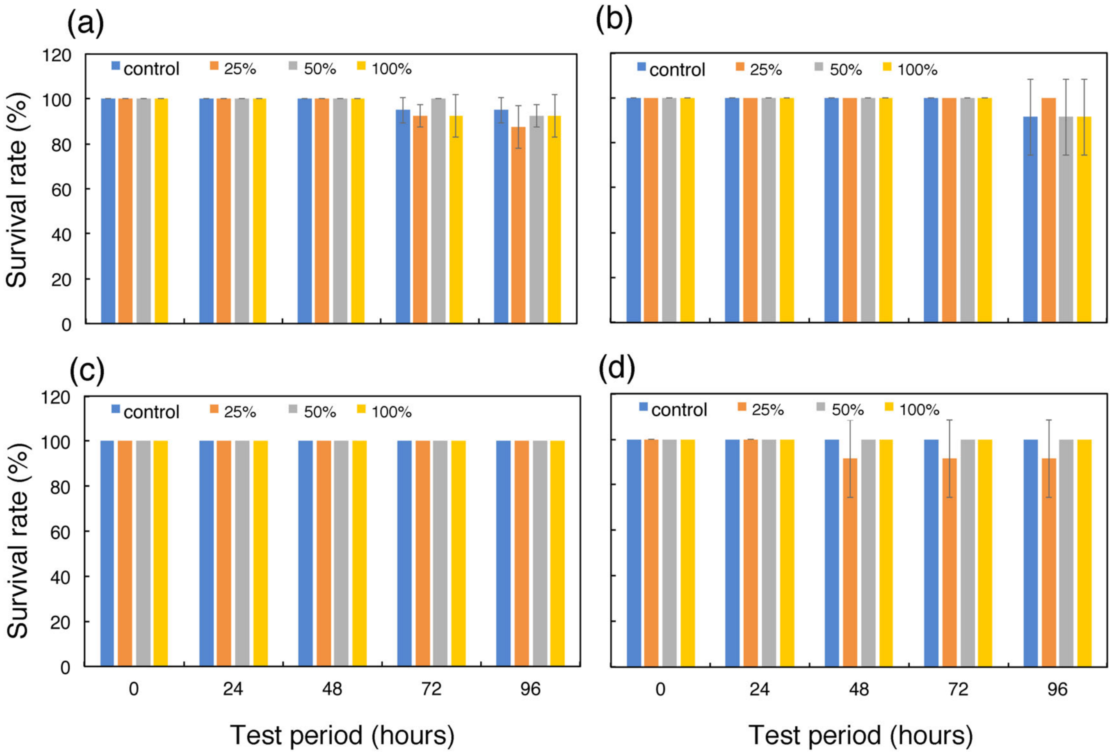

(a)
(b)
(c)
(d)
control
25%
50%
100%
Survival rate (%)
0
20
40
60
80
100
120
0
24
48
72
96
Test period (hours)

Table 1 Comparisons between *O. melastigma* (marine fish) and *O. latipes* (freshwater fish) of lowest-observed-effect concentrations (LOECs) of 5 test chemicals with mortality as the endpoint

Chemical	LOEC value for mortality	
	Oryzias melastigma	*Oryzias latipes*
3-chloroaniline	25 mg/L	25 mg/L
triclosan	300 µg/L	600 µg/L
3,4-dichloroaniline	31 µg/L	62 µg/L
fenitrothion	3.5 mg/L	3.5 mg/L
pyriproxyfen	0.3 mg/L	0.6 mg/L

Table 2 Water quality at each sampling site in Akita harbor and canal

Parameter	Site	year 2019			year 2020
		May	August	October	February
Temperature (℃)	Site A	20.2	27.6	20.1	9.7
	Site B	19.8	25.8	19.8	8.7
pH	Site A	8.08	8.21	7.75	7.86
	Site B	7.91	7.79	7.12	7.38
Salinity	Site A	24	24	24	25
	Site B	8	4	7	2
Conductivity (μS/cm)	Site A	37.7	38.4	39.8	35
	Site B	14.6	6.6	10.2	11.3
Dissolved Oxygen (mg/L)	Site A	5.67	4.51	6.23	5.42
	Site B	5.80	5.32	5.92	5.63