EFFECTS OF ACIDIFICATION ON FISH REPRODUCTION

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ABSTRACT

Acid rain induces the acidification of inland waters which results in damage to aquatic ecosystems that contain fish. In northern Europe and America, where damage by acid rain has been manifested, many populations of fishes have vanished. At present, rapidly expanding industrial activities in Asia have led to a continuous increase in emissions of acidic pollutants, and rain at acidic levels of pH 4 has often precipitated throughout Japan. In order to investigate the effects of acid rain on fish ecosystems and forecast the damages that will be sustained in the future, it is necessary to clarify the biological responses of fish to an acidic environment. Therefore, we have investigated the effects of acid exposure on physiological changes in fishes. Levels of pH 4 constitute acidic conditions lethal to most species of fish. When fish were exposed to low pH, loss of sodium (Na^+) and chloride (Cl^-) ions from the body fluid occurred, resulting in a decrease in plasma osmotic pressure. Dace *Tribolodon hakonensis* inhabiting Lake Osorezan in Japan, which is highly acidic (pH 3.6), had profoundly differentiated gill chloride cells which showed high V-ATPase activity that stimulates the proton pump of the cell membrane to excrete H^+ ions. Moreover, it was found that somatolactin, a pituitary hormone, is possibly involved in the acid-base regulation. Even if pH is not low enough to be lethal, the stress of acidification induces various physiological and ecological problems in fish. When juvenile carp were exposed to pH 4.5, plasma cortisol levels peaked in response to acid stress, and immuno-globulin (IgM) levels subsequently decreased. This result suggests that acid stress depresses the immune system of fish. When mature salmonid fishes are exposed to pH 4.5-5.0, inhibition of development and increases in malformation are observed in the embryos of their offspring. Plasma levels of sex steroids and gonadotropin exhibited abnormally high levels and there was a possibility that acid stress disrupted the endocrine control over reproduction. Additionally, the acidic condition of pH 5.8 completely inhibited the homing migratory behavior of land-locked sockeye salmon, and extremely slight acidification (near pH 6) inhibited their spawning behavior. These results suggest that salmonid fish are highly sensitive to slight changes in acidity and they attempt to avoid an environment perceived to be deleterious to their offspring.

INTRODUCTION

The phenomenon called acid rain results from industrial activities where sulfuric and nitric acid are produced by the release of sulfuric oxides (SOx) and nitrogen oxides (NOx) into the atmosphere. Acid rain induces the acidification of inland waters which results in damage to aquatic ecosystems, including fish. In northern Europe and America, as damage by acid rain has been manifested since the 1960s, many populations of salmonid fishes constituting important fisheries resources have vanished (Schofield 1976). In particular, great numbers of Atlantic salmon *Salmo salar* and brown trout *S. trutta* were destroyed by the acidification induced by the rapid inflow of acid pollutants into rivers during spring snow-melts (snow-melt acid shock) in Scandinavian countries. This phenomenon is called “fish kill” (Leivested and Muniz 1976). Countries in these areas dedicate an enormous percentage of their national budgets to the neutralization of acidification by means of liming (Appelberg et al. 1995).

At present, rapidly expanding industrial activities in Asia have led to a continuous increase in emission of acidic pollutants resulting in rain at acidic levels of pH 4 precipitating throughout Japan (Japan Environment Agency 1997). In order to investigate the effects of acid rain on fish ecosystems and forecast the probable damage that could be sustained in the future, it is necessary to clarify detailed biological responses of fish to an acidic environment in Japan. Therefore, we have investigated the effects of acid exposure on physiological and ecological processes in fishes, especially regarding reproduction.

ACUTE EFFECTS AND ACID TOLERANCE

Levels of pH 4 constitute acidic conditions lethal to fish (Ikuta et al.1992). Fish have the ability to regulate their acid-base balance in order to maintain normal pH of their body fluids under acidic ambiance. When fish are exposed to a low pH, chloride cells in the gill tissue take up bicarbonate (HCO_3^-) ion from the outside to neutralize the hydrogen (H^+) ion flowing in the body. At this time, the loss of sodium (Na^+) and chloride (Cl^-) ions from the body fluids occurs, and plasma osmotic pressure decreases (Fig.1) (Iwata et al. 1990). This process is considered to be one of the major reasons why freshwater fish die under acidic conditions. In tilapia *Orechromis niloticus*, *O. mossambicus*, and medaka *Oryzias latipes*, Na^+,K^+-ATPase activity in chloride cells increases in association with Na^+ loss when exposed to low pH; this suggests that Na^+,K^+-ATPase may act to affect Na^+ uptake under an acidic hypotonic environment (Yada and Ito 1997, 1998).

From these results, it was considered that plasma Na levels could be used as an indicator to estimate the acute effects of acidification on fish. When rainbow trout *Oncorhynchus mykiss* were exposed to various acidic conditions, the fish showed lower plasma Na^+ levels and the Na^+ levels and pH were found to be significantly correlated (Fig.2) (Yada et al. 2000). Thus, we began checking the health of fish in the natural environment by means of a medical blood ion and gas meter (i-STAT Corp. USA).

Dace *Tribolodon hakonensis* inhabiting Lake Osorezan in Aomori Prefecture, Japan, which is highly acidic (pH 3.6), are well-known to be acid tolerant (Satake et al. 1995), whereas the same species inhabiting areas of neutral pH exhibit a low tolerance to low pH. A recent study revealed that dace in Lake Osorezan have especially differentiated chloride cells which enlarge and gather to form a follicle-shaped structure. Since these cells show high V-ATPase activity that stimulates the proton pump of the cell membrane, their function is possibly to excrete H^+ ions which flow into the body from the outside (Fig. 3) (Kaneko 1997). Moreover, it was found

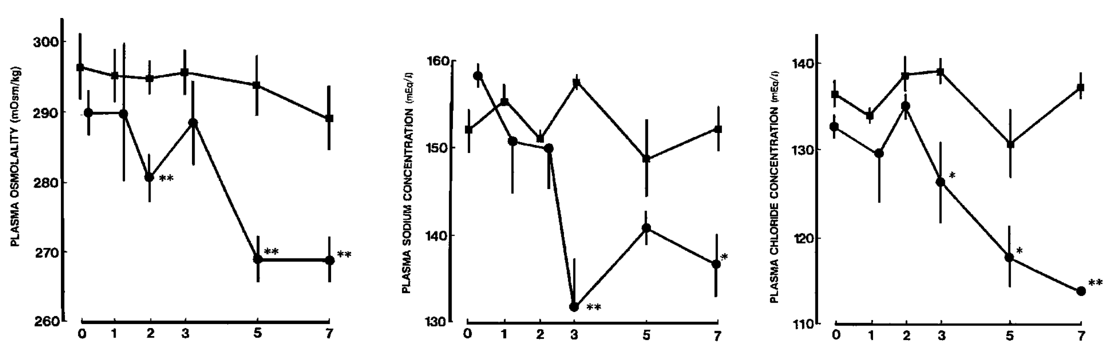

Figure 1. Changes in the plasma osmolality (left), sodium concentration (middle), and chloride concentration (right), in the pH 3.9-exposed (circle) and the control (square) yearling Japanese char. Data are shown as means ± SE (n=6). *; P<0.05, **; P<0.01.

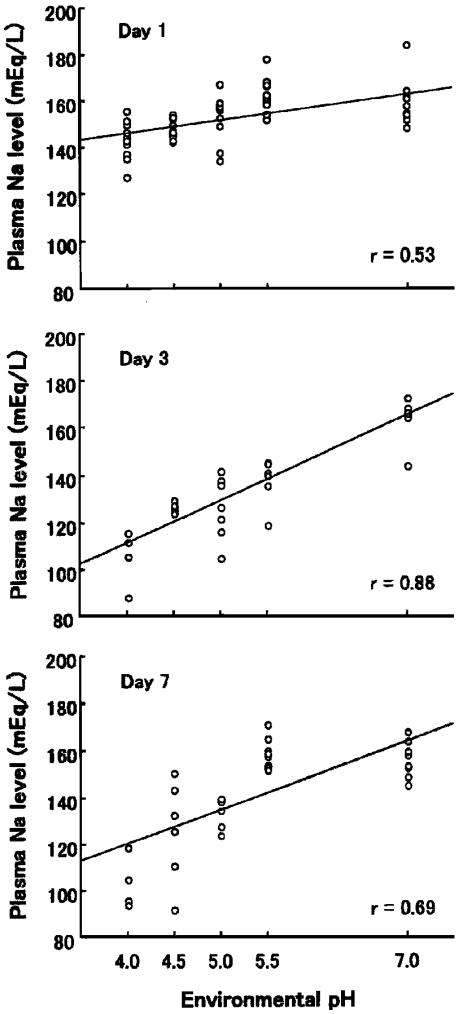

Figure 2. Correlation between the plasma Na levels and environmental pH in rainbow trout on d 1, d 3 and d 7 after acid-exposure.

that somatolactin, a pituitary hormone of the growth hormone and prolactin family, is possibly involved in the acid-base regulation of chloride cells (Kakizawa et al. 1996, 1997).

EFFECTS OF SUB-LETHAL ACIDIFICATION

Even if pH is not at a lethal level, the stress of acidification induces various physiological and ecological problems in fish. As observed under laboratory conditions, chum salmon *O. keta* juveniles could significantly perceive low acidity such as pH 5.8 and displayed avoidance behavior (Ikuta et al. 1996). At this time, juvenile salmon showed a high peak of plasma cortisol which is known as the "stress

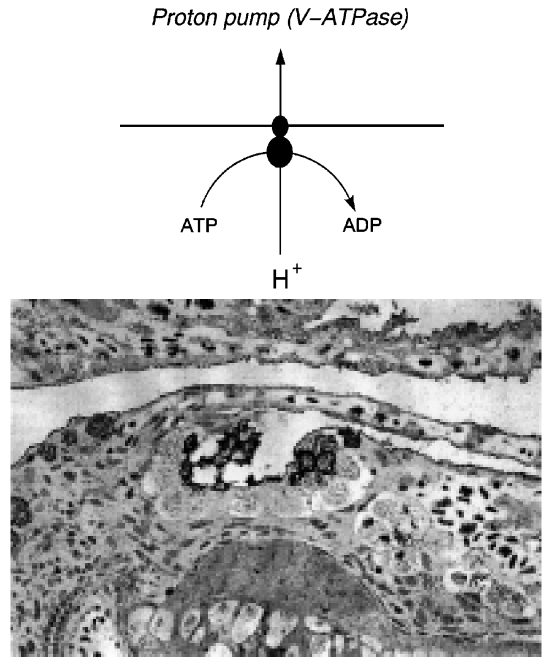

Figure 3. Schematic of proton pump in cell membrane (upper) and cross section of chloride cells positively stained with antibody to V-ATPase in gill membrane of dace in Lake Osorezan (lower).

hormone" secreted from the adrenal cortex. This indicates the possibility that salmon will disappear from the areas where acidification is beginning, due to their instinctive avoidance behavior.
It is well-known that cortisol secreted due to stress reduces immune and reproductive functions in fish (Carragher et al. 1989). When juvenile carp *Cyprinus carpio* were exposed to water of pH 4.5, plasma cortisol levels peaked rapidly in response to acid stress, and immuno-globulin (IgM) levels subsequently decreased (Fig. 4) (Ikuta et al. 1997). IgM is a protein constituting antibody that specifically binds to alien substances such as viruses and bacteria, and prevents infection. This result suggests that acid stress depresses the immune system in fish. Therefore, it is also very important to consider the indirect effects of acidification on physiological mechanisms of fish, even if the acidification itself is not lethal.

EFFECTS ON FISH REPRODUCTION

Recent research has also revealed that sub-lethal acid stress affects reproduction of fish. If mature salmonid fishes are exposed to sulfuric acid water of pH 4.5-5.0, inhibition of

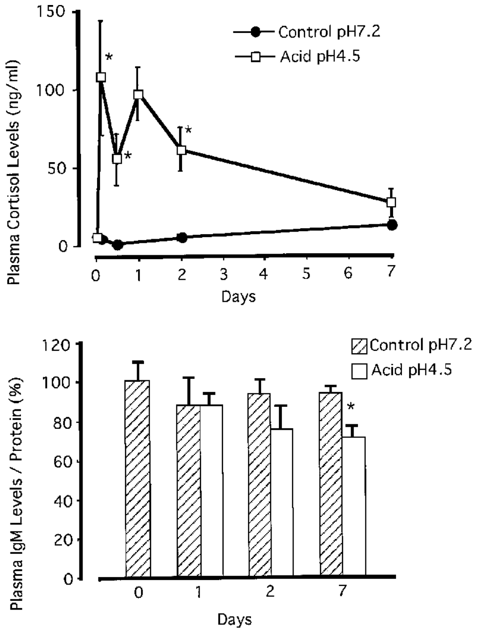

Figure 4. Changes in the plasma cortisol (upper) and immunoglobulin (lower) levels during acid exposure in the juvenile carp. Data are shown as means ± SE. *; $P<0.05$.

development and increases in malformation are observed in the embryos of their offspring (Ikuta and Kitamura 1995). When mature rainbow trout were reared in pH 4.5 just prior to spawning, the eyeing rate (index indicating normal development) of embryos from females exposed to acid decreased drastically, and the malformation rate of embryos produced with sperm from males exposed to acid increased in a time-dependent manner, even if the embryos were cultured in neutral water after fertilization (Fig. 5). As plasma levels of sex steroids and gonadotropin showed abnormally high levels in both male and female fish exposed to acid, there was a possibility that acid stress disrupted the endocrine system of reproduction in fish. In land-locked sockeye salmon *O. nerka*, mature female fish reared at pH 5.0 showed ovulation earlier than did the controls, and the eyeing rates in embryos produced from them rapidly decreased after one wk (Fig. 6). Since the pH 5.0 group showed extremely high plasma levels of 17α20β-dihydroxy-4-pregnen-3-on (DHP), which is a steroid hormone inducing final maturation in oocytes, abnormal oocyte maturation and ovulation might be induced by acid stress (Ikuta et al. 1999). Because similar depression of embryonic development was

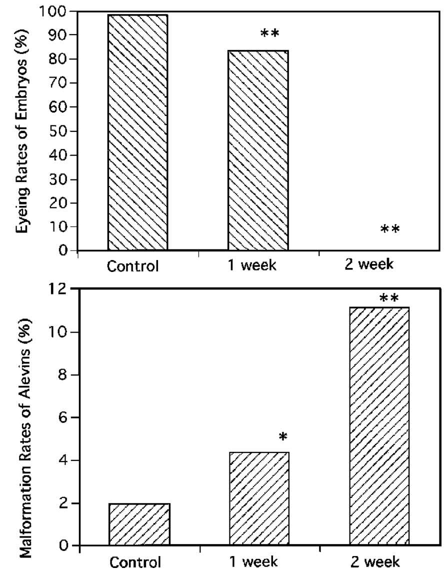

Figure 5. Eyeing rates of embryos produced between the acid-exposed (0, 1 and 2 wk) females and the control males (upper), and malformation rates of hatched alevins produced between the acid-exposed (0, 1 and 2 wk) males and the control females (lower) in rainbow trout. *; $P<0.05$, **; $P<0.01$, compared with the control.

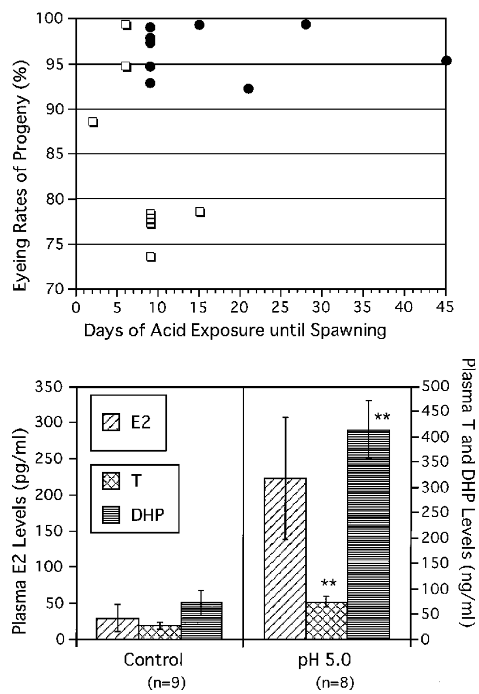

Figure 6. Correlation between days on which pre-ovulatory female sockeye salmon were exposed to pH 5.0 (square) and pH 7.2 (circle) until spawning and eyeing rates of progeny of each individual (upper), and their plasma levels of sex steroids, i.e. estradiol-17β (E2), testosterone (T) and 17α20β-dihydroxy-4-pregnen-3-on (DHP) at spawning (lower). Data of hormone levels are shown as means ± SE. **; $P<0.01$, compared with the control.

reported from female land-locked sockeye salmon, called kokanee in North America (Parker & Mckeown 1987), exposed to pH 5.6, this species is considered very sensitive to acidity especially with regard to reproduction.

Additionally, it has been clarified that slight acidity (in the range of pH 6) is sufficient to depress reproductive behavior of salmonid fish. It is common knowledge that salmonid fishes exhibit homing or migration behavior in which they return to their native rivers for spawning. In observational experiments using artificial two-way channels in which water of neutral and various pH was allowed to flow, acidic conditions such as pH 5.8 completely inhibited upstream migratory behavior of land-locked sockeye salmon, and all of the salmon selected the neutral channel to begin migration. Female adult salmon also show digging behavior by forming a nest on the gravel bottom of the river; this is followed by mating and spawning behavior (Fig. 7). In tank observations, very slight pH changes from 6.8 to 6.4 and less, significantly inhibited their spawning behavior (Fig. 8) (Kitamura and Ikuta 2000). These results suggest that salmonid fish are very sensitive to slight acidity and avoid an environment perceived to be deleterious to their offspring.

CONCLUSION

In the series of studies presented, it has been clarified that acidification of inland waters induced by acid rain or other acidic pollutants

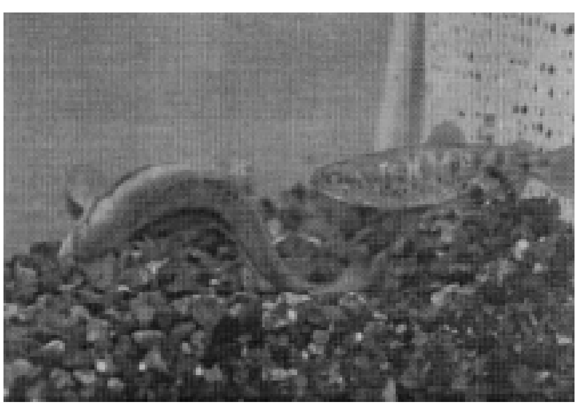

Figure 7. A female salmon showing digging behavior by forming a spawning nest on the gravel bottom (left) and a male fish attending the female in an observation tank.

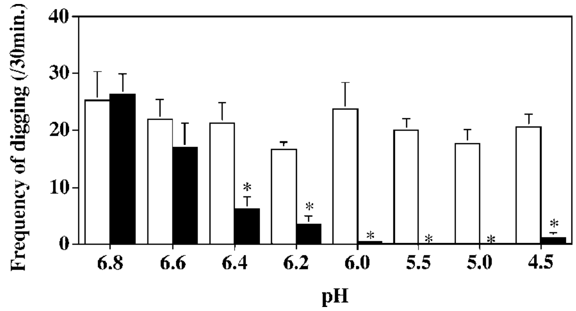

Figure 8. Changes in the frequency of digging behavior of female sockeye salmon. White bars indicate the mean frequency in neutral water (pH 6.8) and dark bars indicate that after transferred to each pH (6.8-4.5). Vertical lines indicate SE. *; $P<0.05$.

causes various physiological and behavioral impediments in fish, even if the acidity is not at a lethal level. Summarizing the results obtained in various experiments using salmonids, such as sockeye salmon, the following scenario in association with inland-water acidification can be proposed in fish (Fig. 9):

1. Extremely slight acidification at pH 6 and lower ranges inhibits homing migration and/or spawning behavior.
2. Sub-lethal acid stress at pH 5 and lower ranges stimulates avoidance of acidic areas or induces failure of immune and reproductive functions via the alteration of physiological mechanisms, including the actions of endocrine factors.
3. Acidification in the range of pH 4 rapidly affects the acid-base regulatory function of gill chloride cells, resulting into mortality due to the efflux of NaCl from body fluid.

At present, the chronic acidification of inland waters by acid precipitation, as seen in North America and Europe, has not yet been observed in Japan, with the exception of volcanic or mineral acid rivers and lakes. However, evidence of potent acid rain has often been observed. Even if the acidity of the water is very slight or at sub-lethal levels, it may adversely affect the physiological functions of immunity and reproduction. Additionally, it is a surprising result that even extremely slight changes in acidity (pH 6) is enough to inhibit instinctively programmed behavior such as homing and spawning in the salmon. Therefore, there is the possibility that the

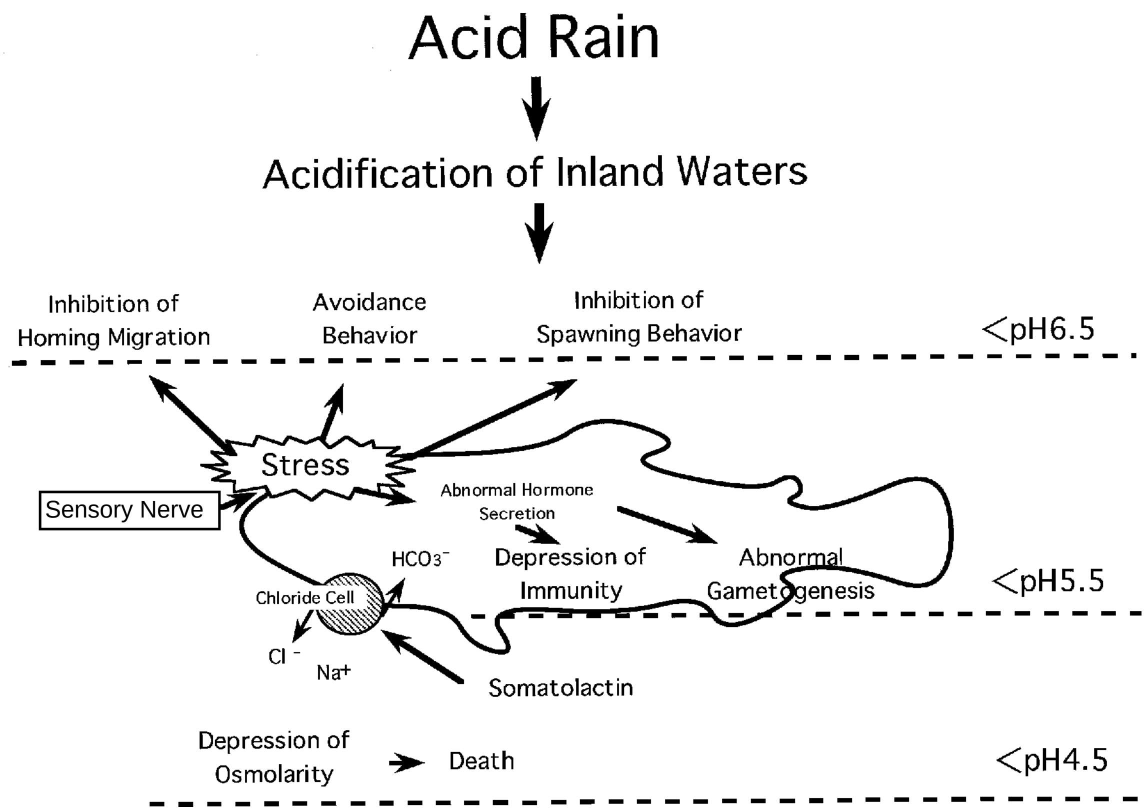

Figure 9. Summary of the effects of water acidification on physiology and behavior of fish.

effects of acid rain will be strongly manifested in the near future in Japan as well. Although we formally used toxicity tests or physiological examinations to evaluate the effects of pollution on organisms, these results suggest that behavioral-specific responses to environment changes are also very important in this kind of research. This is because the inability of fish to reproduce is equivalent to the avoidance of reproductive behavior, even if fish are physiologically capable of doing so.

ACKNOWLEDGMENTS

The authors express gratitude to Dr. Kenichi Satake, Team Leader of Research Project “Studies on the Impacts of Acidic Pollutants on the Life-Environmental Systems,” National Institute for Environmental Studies, for his encouragement and advice. We thank Professor Emeritus Tetsuya Hirano, University of Tokyo, for his instruction in the study of dace in Lake Osorezan. We also thank Mr. Toshio Shikama and Mr. Hidefumi Nakamura, Nikko Branch, National Research Institute of Aquaculture, for their assistance in the experiments, Dr. Masafumi Amano, Kitasato University, and Dr. Arimune Munakata, University of Tokyo, for their assistance in the measurement of hormones, and Dr. Marcy N. Wilder, Japan International Research Center for Agricultural Sciences, for her comments regarding the manuscript. This study was partly supported by the Global Environment Research Promotion Fund, Japan Environmental Agency.

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