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Ecological studies on medaka in a remained habitat in Qinhuangdao, Hebei, China

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Abstract Ecological studies were carried out in the remaining habitat for medaka (*Oryzias latipes*), a marsh in the suburbs of Qinhuangdao City, Hebei Province, China. Sewage released from villages increases the nutrient levels in open water areas of the marsh, while in cattail (*Typha angustifolia*) and reed (*Phragmites communis*) beds the nutrient levels are decreased. There are fewer zooplanktons in the cattail and reed beds than in the open water areas. Sampling fishes with trap nets showed that medaka lived in dense populations in the cattail and reed beds, while the other three fish species, wild goldfish (*Carassius auratus*), topmouth gudgeon (*Pseudorasbora parva*), and mud loach (*Misgurnus bipartitus*), occupied the open water areas in the marsh. A little amount of paradise fish (*Macropodus chinensis*) was also found in the marsh. Japanese freshwater shrimps (*Macrobrachium nipponense*) were found only in the influx canal from waterworks. Indoor aquarium experiments showed that the wild medaka in this marsh was easily cultured with commercial feed similar to domesticated strains. Some ecological aspects of the medaka were discussed based on the observations of the fish in the marsh.

Keywords medaka, *Oryzias latipes*, endangered fish, habitat, ecology

1 Introduction

Medaka (*Oryzias latipes*), also called Japanese rice fish, is one of the most important experimental animals used in basic biological studies and environmental monitoring, as the fish is hard, easily cultured and reproduced in indoor aquarium. Fundamental biological studies using medaka as

a material have been published steadily in recent years (Inoue and Takei, 2003). Even more, medaka was sent in the space shuttle Columbia in 1994 for a space biological research.

Medaka used to be a common fish in rice paddies, streams, lakes, and reservoirs in most regions in China. Being a tiny (2.5–3.5 cm body length for adult fish) and economically unimportant fish, medaka has been given little attention in China. As a result, reports concerning the fish are scarce, and there are almost no special publications on medaka resources and ecology in China. In recent years, the fundamental studies using medaka as a material, particularly in genetics and toxicology, and the monitoring of the water environment with the fish increased, but the medakas in China are mostly Japanese inbred strains, which have problems in strain uncertainty and considerable degeneration.

Apart from its use as model experimental animal, the medaka has been a pet or ornamental fish since the 17th century, and various strains have been cultured in Japan (Yamamoto, 1975). In Taiwan, China, the medaka is also a popular aquarium fish and is given much attention for its natural resource and protection. Recently, in mainland China there also developed a hobby to rear domestic wild fishes, including the medaka, in the garden and aquaria. However, having been neglected for quite a few decades, the medakas have proven to be almost extinct in most of their original habitats in China. Therefore, *Oryzias latipes* was added to the “China Species Red List” in 2005, when the fish was listed as a distinct species in Guangzhou. In Japan, the medaka was categorized as an endangered species in the Japanese Red Data Book in 2003 (Kume and Kubo, 2005). In Taiwan, China, the medaka was thought to have died out for 70 years, before it was rediscovered in only two restricted regions at the end of the last century. In order to restore the medaka natural resources, and establish our domestic experimental medaka stains, knowledge of ecology of the fish is necessary. Therefore, we have made continuous ecological investigations on the medakas in the remaining habitat in Qinhuangdao, Hebei Province for two years (2006 and 2007).

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2 Methods

2.1 Investigational sites

In the west suburb of Qinhuangdao city, there is a marsh with an area of approximately 15 hectares, which is surrounded by a small earthen road, two railroads, warehouses and a village. The marsh receives wastewater from the waterworks in the north and the village in the west. Along the south side of the marsh, there is a small dam with a single concrete pipe (2 m in diameter) underneath, through which the water passes via an approximately 1.5 km long canal leading to the sea. Seawater can never reach the marsh, even at the highest tides. With a constant water supply from these wastewater sources, the marsh never dries up, with only some water-level fluctuations. Cattail (*Typha angustifolia*), and also reed (*Phragmites communis*) in some regions, grow throughout the marsh, except for the central area where the water is deeper. During the summer, common duckweed (*Lemna minor*) covers almost the entire surface of the marsh. The marsh ices up in early December and thaws in the middle of March. There were a total of 9 investigational sites selected, with 3, 3, 1, 1 and 1 located on the east bank, on the west bank, the wastewater canal from the waterworks, the wastewater canal from the village, and the efflux canal leading to the sea, respectively.

2.2 Sampling and measurement

At each investigation site, samples were taken regularly to investigate the water physicochemical parameters, zooplanktons and fishes except in winter when the marsh was frozen.

Water samples were taken using 1-liter plexiglass water-samplers (purchased from the Institute of Hydrobiology, Academy of Sciences, China). Temperature and dissolved oxygen (DO) of the water were determined *in situ* immediately, while other physicochemical parameters (pH, salinity, chemical oxygen demand (COD), ammonium nitrogen, nitrites, nitrates, and hydrogen sulfide) were determined within 24 h in our laboratory.

Zooplanktons were sampled with plankton nets (# 13, produced by the Institute of Hydrobiology, Academy of Sciences, China), stored in plastic bottles, and then identified in the laboratory.

Trap nets (3 mm × 3 mm mesh) were placed at depths of 0.5 m and 1 m at each investigation site, aquatic plants (cattails and reeds) were cut off to settle the nets when necessary. The fish and other animals caught in the trap nets were taken out and brought to the laboratory for identification and measurements. All data of the samples were initially processed with one way analysis of variances (ANOVA), followed by *t*-test or *q*-test with proper degrees of freedom.

2.3 Indoor aquarium culture

In early April of 2007, live medakas caught by the trap nets were placed in plastic bags filled with *in situ* water and pure oxygen, and then brought to the laboratory. The fish were cultured in eight 100 L aquaria filled with tap water in the laboratory at a density of 200 fish per aquarium. The fish in four aquaria fed on the lower parts of the stems of cattail and reed cut from the marsh, which were renewed weekly, while in the other four aquaria, the fish was fed daily on commercial fish diets at a rate of approximately 5% of body weight. The behavior of the fish was observed daily.

3 Results

3.1 Water physicochemical parameters

There were significant differences in water physicochemical parameters at different investigation sites (Table 1). The samples at the influx wastewater canal from the waterworks showed proper water quality, while those from the village showed high levels of COD and nitrogen but low levels of DO, and kept to a less extent at the efflux canal leading to the sea. At the investigation sites along the east and west banks of the marsh, the levels of COD and nitrogen remarkably decreased but the level of DO increased compared with those at the influx sewage canal and efflux canal.

Salinities at all the investigation sites were almost the same, ranging from 0.5 ppt to 2.0 ppt in spring and autumn, but 0–0.3 ppt in summer.

Water temperatures were approximately 12°C in early April, which gradually increased to a maximum of 28°C in August (in open water), and then fell to 10°C in October. During summer, water temperatures in the sites where the plants grew thick were approximately 1–2 degrees lower than those in open waters of the marsh.

3.2 Zooplanktons

Rotifers (*Brachionus calyciflorus*) and copepods (*Cyclops* sp. and *Mesocyclops leuckarti*) were observed in early April. Water flies (*Daphnia magna* and *D. pulex*) appeared in the middle of May and became predominant from June to September, forming massive clusters on the surface in the morning. From October, all the zooplanktons decreased gradually, till November when only rotifers were left with low density. In cattail and reed beds, all the zooplankton densities were remarkably lower than those in the open waters of the marsh.

3.3 Trap net samplings

Animals caught by trap nets in the marsh consisted of medaka (*Oryzias latipes*), wild goldfish (*Carassius*

Table 1 Water physicochemical parameters in various investigation sites

parameter	wastewater from waterworks	wastewater from the village	west banks of the marsh	east banks of the marsh	canal leading to the sea
DO	8–12, Apr. –May (a)	0–3, Apr. –May (b)	3–7, Apr. –May (b)	5–12, Apr. –May (a)	2–5, Apr. –May (b)
/(mg · L ⁻¹)	5–10, Jun. –Aug. (a)	0–2, Jun. –Aug. (b)	3–5, Jun. –Aug. (b)	3–14, Jun. –Aug. (a)	1–4, Jun. –Aug. (b)
	7–11, Sept. –Nov. (a)	0–3, Sept. –Nov. (b)	4–8, Sept. –Nov. (a)	7–11, Sept. –Nov. (a)	2–6, Sept. –Nov. (b)
COD	10–29, Apr. –May (a)	146–266, Apr. –May (b)	32–75, Apr. –May (b)	14–30, Apr. –May (a)	80–125, Apr. –May (b)
	5–22, Jun. –Aug. (a)	154–320, Jun. –Aug. (b)	49–113, Jun. –Aug. (b)	25–50, Jun. –Aug. (a)	130–214, Jun. –Aug. (b)
	12–24, Sept. –Nov. (a)	100–278, Sept. –Nov. (b)	40–94, Sept. –Nov. (b)	23–40, Sept. –Nov. (a)	80–137, Sept. –Nov. (b)
pH	7.6–8.1	6.7–7.4	7.0–7.6	7.5–8.2	7.0–7.8
NH ₄ ⁺ + NH ₃	0–0.4, Apr. –May (a)	0.8–1.8, Apr. –May (b)	0.3–1.2, Apr. –May (b)	0.1–0.5, Apr. –May (a)	0.3–0.9, Apr. –May (a)
/(mg · L ⁻¹)	0.2–0.6, Jun. –Aug. (a)	1.9–4.6, Jun. –Aug. (b)	1.0–2.9, Jun. –Aug. (b)	0.3–0.8, Jun. –Aug. (a)	1.0–2.9, Jun. –Aug. (b)
	0.2–0.6, Sept. –Nov. (a)	0.8–2.8, Sept. –Nov. (b)	0.7–1.9, Sept. –Nov. (b)	0–0.6, Sept. –Nov. (a)	0.6–1.1, Sept. –Nov. (b)
NO ₃ ⁻	0.2–0.4, Apr. –May	0.5–0.9, Apr. –May	0–0.7, Apr. –May	0.4–0.9, Apr. –May	0–0.6, Apr. –May
/(mg · L ⁻¹)	0.1–0.5, Jun. –Aug.	0–0.4, Jun. –Aug.	0–0.3, Jun. –Aug.	0–0.6, Jun. –Aug.	0–0.9, Jun. –Aug.
	0.2–0.5, Sept. –Nov.	0.5–1.0, Sept. –Nov.	0–0.8, Sept. –Nov.	0–1.0, Sept. –Nov.	0–0.9, Sept. –Nov.
NO ₂ ⁻	0, Apr. –May (a)	0.0–0.03, Apr. –May (b)	0.01–0.02, Apr. –May (b)	0, Apr. –May (a)	0.01–0.02, Apr. –May (b)
/(mg · L ⁻¹)	0, Jun. –Aug. (a)	0.01–0.04, Jun. –Aug. (b)	0.01–0.02, Jun. –Aug. (b)	0, Jun. –Aug. (a)	0.01–0.02, Jun. –Aug. (b)
	0, Sept. –Nov. (a)	0.01–0.04, Sept. –Nov. (b)	0–0.02, Sept. –Nov. (b)	0, Sept. –Nov. (a)	0.01–0.02, Sept. –Nov. (b)
H ₂ S	0, Apr. –May (a)	0.02–0.05, Apr. –May (b)	0–0.03, Apr. –May (b)	0, Apr. –May (a)	0.01–0.04, Apr. –May (b)
/(mg · L ⁻¹)	0, Jun. –Aug. (a)	0.03–0.07, Jun. –Aug. (b)	0.03–0.06, Jun. –Aug. (b)	0, Jun. –Aug. (a)	0.01–0.07, Jun. –Aug. (b)
	0, Sept. –Nov. (a)	0.02–0.05, Sept. –Nov. (b)	0–0.04, Sept. –Nov. (b)	0, Sept. –Nov. (a)	0.02–0.04, Sept. –Nov. (b)

Note: Different letters are significantly different at 0.05 probability level.

auratus), topmouth gudgeon (*Pseudorasbora parva*), mud loach (*Misgurnus bipartitus*), paradise fish (*Macropodus chinensis*) and Japanese freshwater shrimp (*Macrobrachium nipponense*). At the influx sewage canal there was no yield from trap nets in any season in 2006 and 2007. However, at the influx canal from the waterworks the net yield was composed of wild goldfish, topmouth gudgeon, mud loach and Japanese freshwater shrimp, but no medaka. The trace amount of paradise fish was found in the west side of the marsh. A large amount of the medakas were caught in the dense cattail and reed beds, with a smaller amount of the medaka at the sites where loose cattail and reed grew, but no medaka in open water areas was found (Table 2).

3.4 Indoor aquarium culture

Medaka caught from the marsh (2.31 ± 0.42 cm in body length) started to feed from the next day on after they were settled in indoor aquaria. The fish actively ate both feed particles on the surface and feed pellets at the bottom. After one week, some male medakas started to occupy territory

and afterwards some female medakas carried eggs at their anal fins. In the four aquaria where cattail and reed stems were placed and renewed without fine feed used, the fish actively scraped debris on the plant stems and at the bottom. The beginning of territorial behavior and the appearance of eggs at the anal fins of female fish were approximately 2–4 days later than the fish fed with commercial feed.

4 Discussion

Medaka is amphidromous, and moves between salt and fresh water at some points in its life. However, there are no literatures available to indicate at which particular period in its life cycle the fish migrates between the two media. Though the marsh we investigated connects to the sea, our samplings did not show the medakas nor the other two common local diadromous fishes, common sea perch (*Lateolabrax japonicus*) and So-iuy mullet (*Mugil soiuy*), in the canal leading to the sea, perhaps because the poor water quality in the canal did not allow the migration of the

Table 2 Yields (individuals per net) from the trap nets at different investigational sites

investigational sites	medaka	top mouth gudgeon	wild goldfish	mud loach	paradise fish	Japanese shrimp
canal from the waterworks	0	5.7±2.3	10.7±2.5	3.6±2.1	0	10.6±4.1
sewage canal from the village	0	0	0	0	0	0
west side, plants loose	0	3.8±1.4	2.3±0.8	15.3±4.8	0	0
east side, plants dense	34.5±6.4*	2.4±0.9	0.6±0.3	0	1.4±0.8	0
east side, plants loose	3.5±1.1	23.8±5.4*	9.6±2.3	9.8±5.2	0.4±0.1	0
canal leading to the sea	0	4.2±2.6	6.3±1.5	5.8±2.3	0	0

Note: Data were obtained from 12 May to 6 June, 2007. Similar trend of data was also obtained in the corresponding time in 2006 (Mean±SD; * means significant difference at 0.05 probability level).

fishes. The water in the investigated marsh where the medaka lives is brackish; however, our successful freshwater culture in indoor aquaria and successful freshwater pond culture in Kunming of Yunnan Province (unpublished) both seem to confirm that seawater or brackish water is not indispensable for this race or strain of medaka.

The water in the marsh is eutrophic, with high levels of COD, nitrogen and hypoxia, especially in summer. Sewage released from the village formed an almost dead zone (where only hypoxia-tolerant water fleas live) along the current from the sewage canal through the open water of the marsh to the efflux canal leading to the sea. However, extensive cattail and reed beds in the marsh obstructed the sewage current, and further, improved the water quality by absorption of nutrient substances, so that at the investigational sites where the plants grew thick and situated far away from the sewage current had significantly lower COD and nitrogen levels but higher DO levels based on observations.

The extensive cattail and reed beds not only functioned as water treatment, like the constructed wetland in water treatment for eutrophication, but also supplied a special ecological niche for the medaka. The capture of medaka from the trap nets showed a close correlation not only with the water quality, but also with the plant density. On the west side of the marsh where the cattails and reeds were extensive and dense, a majority of fishes captured in the net were medaka, with only small percentage of the other three fishes (topmouth gudgeon, wild goldfish and paradise fish). It needs to be noted that the other three fish species caught in these regions of the marsh were unexceptionally juveniles with body lengths of less than 3 cm. The cattail and reed beds seemed to serve as sieves that sorted out all the aquatic animals bigger than approximately 3 cm in body length, which is exactly the maximum size for adult medakas. In contrast, at the investigational sites where cattail and reed plants were loose and in open waters of the marsh, Topmouth gudgeon, wild goldfish and mud loach comprised most of the net yields. Obviously, the medaka prevailed in thick and extensive cattail and reed beds or in the special ecological niches in the marsh, while the other three fishes occupied the open water regions of the marsh. Topmouth gudgeon, wild goldfish and mud loach are

common fishes in eutrophic natural waters throughout China, and also common pests in fish ponds. Japanese freshwater shrimp, a species commonly found in the vegetated waters throughout China, was restricted to the influx canal from the waterworks, which clearly indicated that the shrimp is less competitive than the medaka in cattail and reed beds, as well as less competitive than the three fish species (topmouth gudgeon, wild goldfish and mud loach) in the open water areas in the marsh. The trace amount of paradise fish in the marsh is of little ecological importance.

The sewage released elevated nutrients in the water of the marsh, resulting in dense zooplanktons, such as rotifers, copepods, cladocerans, mosquito larvae, etc. The density of zooplanktons was remarkably lower in cattail and reed beds than that in the open water areas; perhaps because there were more inhabiting fishes, lower nutrient levels resulting from plant absorption, and less sunlight available with the shading. Our indoor aquarium culture showed that organic debris on the plants and at the bottom were also main foodstuffs for the medaka, which may give the explanation for why a considerable amount of medakas were held in the niches with limited zooplankton. Our experiment also confirmed that this race or strain of wild medaka can be readily cultured in aquaria with commercial feed, similar to domesticated strains (Huang, 1988). The medaka in this marsh tried to replace degenerated strains in some academic institutes. Our pilot pond culture of this wild medaka was carried out in both north and south China.

Our investigation in the marsh may represent the characteristics of the remaining habitats for medaka in China, wetlands with dense aquatic vegetation. Other original habitats for medaka, rice paddies, streams, rivers, lakes and so on, were almost found deprived of this fish species. However, wetlands have been rapidly disappearing with the development of the urban and rural economy in China, except for some unexplored regions and conservation zones. Widespread use of insecticides, herbicides and fertilizers have made the original main habitats and rice paddies no more inhabitable to medaka. Only the recently developed organic rice culture and traditional co-culture of rice and fish leave opportunity for

medaka to re-inhabit. The runoff of insecticides and herbicides probably killed the medaka in some natural waters. Various pollutants in waters allowed tolerant aquatic animals to invade and out-compete the original medaka inhabitants. A good example is that mosquitofish (*Gambusia affinis*) has replaced almost all medakas in their original habitats in south China (Xie et al., 1996; Chen, 2002). Mosquitofish, a native fish in South America, was introduced to many countries including China for mosquito control. In most countries where mosquitofish have been introduced, it has been proved that the endemic fish species were already providing maximal mosquito control, and that the introduction of mosquitofish has been both unnecessary and highly damaging to endemic fish and other endemic aquatic life (mosquitofish, In: wikipedia the free encyclopedia, <http://en.wikipedia.org/>). In south China, mosquitofish, with their extraordinary survival ability and tolerance to nearly all pollutants, prevailed over the medaka and threatened local amphibians. The situation can hardly be reversed as the mosquitofish shares the same ecological niches with medaka (Chen, 2002), but may be improved if pollution is properly controlled, as Wada et al. (1974) found that *Gambusia affinis* inhabited in the more or less sewage polluted waters in urbanized regions, while *Oryzias latipes* in cleaner waters in rural regions in the Tokyo Metropolitan area. The chilly winter in north China can impede further invasion of mosquitofish, thus the more or less intact north Chinese medaka races, with the northmost record in Nunjiang River (Zhang, 1995) and a new record in the northwest China (Zhao, 2006), are promising for propagation and exploitation. Thorough ecological investigations and studies on medaka should be carried out before the procedure for restoration of this fish is made.

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