

# Effects of pentachlorophenol (PCP) on the oxygen consumption rate of the river puffer fish *Takifugu obscurus*

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**ABSTRACT:** Laboratory bioassays were conducted to determine the effects of pentachlorophenol (PCP) on the oxygen consumption of the river puffer fish *Takifugu obscurus*. Oxygen consumption of 6 to 9 mo old puffer fish was measured with an automatic intermittent-flow-respirometer (AIFR). Oxygen consumption rate was significantly increased by exposing fish to concentrations of 50, 100, 200 and 500 ppb PCP. Following exposure to 50 or 100 ppb PCP, the instantaneous rate of oxygen consumption was considerably increased. *T. obscurus* exposed to 200 ppb PCP, however, exhibited a breakdown in the biorhythm of oxygen consumption presumably due to a strong physiological stress caused by the higher PCP concentrations. River puffer fish exposed to 500 ppb PCP died in 10 h.

**KEY WORDS:** PCP · Oxygen consumption · River puffer fish *Takifugu obscurus*

## INTRODUCTION

Pentachlorophenol (PCP) is a widely used biocide (Cirelli 1978), even though it is known to be highly toxic to most living organisms. Samis et al. (1993) reported that growth rate of bluegills *Lepomis macrochirus* was significantly reduced at a PCP concentration of 48 ppb. Its primary mode of action appears to be the uncoupling of mitochondrial oxidative phosphorylation at low concentrations (Bostrom & Johansson 1972) and complete inhibition of the electron transport chain at higher concentrations (Desaiyah 1978).

Toxic chemicals may cause damage to the gill membranes and affect the physiological functions of the fish in variety of ways (Jobling 1994). Toxic compounds are accumulated in animal organs, especially in the liver of fish, where accumulated concentrations can be more than 1000-fold higher than in the environment (Freitag et al. 1982, Gluth et al. 1985). Palmer (1995) stated that

one way to decipher the bio-physico-chemical linkage would be to test the effects of a variety of chemicals on organism biorhythms, i.e. how they alter their periods or phase. Despite the well-documented role of PCP in biochemical responses (Coglianese & Jerry 1982, Yousri & Hanke 1985, Castren & Oikari 1987) and ecological processes (Whitney et al. 1981, Kierstead & Baerlocher 1989, Freedman 1995), its effects on metabolic activities and biological rhythm of oxygen consumption of fish have not been well described.

The river puffer fish is endemic to Korean and Chinese waters (Chyung 1977), and known to spawn in spring in the rivers and migrate to the South China Sea in late autumn for wintering. This species is one of the most favored food fish and thus has been caught in significant quantities from most estuaries and freshwater habitats.

This paper deals with the direct effect of PCP on oxygen consumption of the river puffer fish measured by a specially designed automatic intermittent-flow-respirometer (AIFR).

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## MATERIALS AND METHODS

**Animals.** The river puffer fish *Takifugu obscurus* used in the experiment were reared in a culture tank (100 l) for 6 to 9 mo after artificial insemination at the Korea Ocean Research and Development Institute (KORDI). The 20 fish [total length =  $6.0 \pm 0.1$  cm (mean  $\pm$  standard error,  $\bar{x} \pm$  SE),  $1.05 \pm 0.04$  g dry wt ( $\bar{x} \pm$  SE)] were acclimatized to the experimental temperatures of 15°C over 4 wk.

**Experimental design.** For measurement of oxygen consumption rate in a control (no PCP) and at 4 different PCP concentrations, 4 *Takifugu obscurus* were put into a 1.4 l experimental chamber at a time. The fish were starved during the experiments in order to exclude a rise in oxygen consumption due to feeding and digestion. The sea water (30.3 to 31.1‰) was filtered free of bacteria through sterile membrane filters (2 Sartorius capsule filters, input 0.2  $\mu$ m and output 0.07  $\mu$ m, membrane pump, KNF, Neuberger, Germany) to reduce background oxygen consumption and to prevent the buildup of a microbial film in the apparatus (which was also thoroughly cleaned between experimental runs). Oxygen consumption rate was calculated from the changes in oxygen saturation level in the test chamber with time. The saturation concentration  $K_{O_2}$  (ml l<sup>-1</sup>) was calculated for standard conditions (atmospheric pressure  $P_{atm} = 1$  atm = 1013 mbar) as a function of temperature and salinity using the formula of Weiss (1970):

$$\ln K_{O_2} = A_1 + A_2(100/T) + A_3 \ln(T/100) + A_4(T/100) + S[(B_1 + B_2(T/100) + B_3(T/100)^2)]$$

where  $T$  is temperature (K) and  $S$  is salinity (PSU) at the time of measurement, and  $A$  and  $B$  are the following constants:  $A_1 = -173.4292$ ;  $A_2 = 249.6339$ ;  $A_3 = 143.3483$ ;  $A_4 = -21.8492$ ;  $B_1 = -0.033096$ ;  $B_2 = 0.014259$ ;  $B_3 = -0.0017000$ . To obtain the concentration in mg l<sup>-1</sup>, the following conversion of gas volume  $V_{std}$  under standard conditions into the gas volume  $V_R$  under measured conditions was used:

$$V_R = V_{std}(1013/P_{atm})(T/273.15)$$

with  $T$  (K) and  $P_{atm}$  (mbar) being taken at the time of measurement (Mortimer 1983). Following this,  $K_{O_2}$  (mg l<sup>-1</sup>) was calculated (Forstner & Gnaiger 1983):

$$K_{O_2} \text{ (mg l}^{-1}\text{)} = K_{O_2} \text{ (ml l}^{-1}\text{)} \times 1.429$$

The following points should be observed to obtain a better result when an AIFR is used: (1) accumulation of excretory products should be avoided; (2) measurements should be conducted over long periods of time; (3) the oxygen tension during measurements should be kept above 86%; (4) human intervention and disturbances should be avoided.

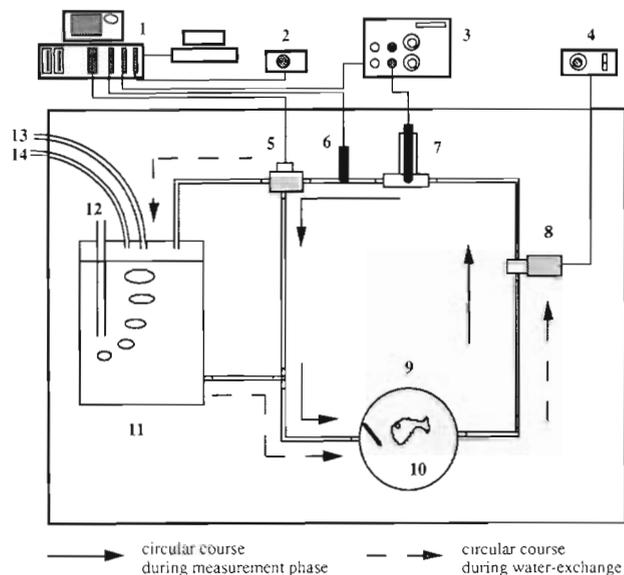


Fig. 1. Schematic (not to scale) of apparatus used to measure oxygen consumption in the river puffer fish *Takifugu obscurus*. 1: Computer for control and data storage; 2: air pressure sensor; 3: picoammeter; 4: pump control; 5: 3-way valve; 6: temperature sensor; 7: oxygen sensor; 8: toothed wheel pump; 9: chamber; 10: fish (test animal); 11: reservoir container; 12: air supply; 13: air exhaust pipe; 14: PCP input

With due regard to these points, the AIFR was specially designed (Fig. 1) by modifying that of Dorrien (1993) using much-improved software for the present study. Oxygen saturations were recorded at every second by the digital controlling unit through a picoammeter and the calculation of mean oxygen consumption displayed graphically every 90 s. When the oxygen saturation dropped below the designed limit, the magnetic drive gear pump and 3-way magnetic valve (332F, Nortec, Germany) supplied the system with oxygen-saturated seawater until the selected oxygen saturation was reached. The magnetic drive gear pump (MS-Z, Ismatec SA, Switzerland) was installed horizontally and produced a water flow rate of about 690 ml min<sup>-1</sup>. Oxygen saturation levels in the experimental chamber were always maintained between 95% (highest) and 86% (lowest). The measuring system was installed in an experimental incubator (MLR-350, REVCO, USA) with constant temperature of 15°C and in darkness. Salinity was measured with a salinometer (LF 320, WTW, Germany); water temperature and air pressure were continuously measured during the experiments using a thermometer (Pt-100, Farnell, Germany) and a barometer (Sensym-Hs 20, Farnell, Germany), respectively; and the oxygen probe (Eschweiler-15  $\mu$ PO<sub>2</sub>, Germany) was calibrated before the experiment began. The whole experiment was controlled automatically by a computer program. After

each experiment, the chamber was rinsed with oxygen-saturated water and the probe voltage examined to ascertain whether it had deviated from the gauge voltage at the beginning of the experiment. For reference, this voltage was subsequently tested between 6 and 24 h by measurement of the oxygen consumption in the chamber without any *Takifugu obscurus* present. No measurements were made while flushing the chamber with oxygen saturated seawater from a storage tank (10 l) to restore the upper oxygen saturation level to 95%.

**Pollutants.** The PCP stock solutions were prepared using reagent grade PCP (P-1045, Sigma Chemical Co.) at concentrations of 50, 100, 200, and 500 ppb. PCP solutions were injected through small holes cut on the cap of the reservoir container (see Fig. 1). *Takifugu obscurus* were exposed to PCP for ca 120 h; this was found to be long enough to allow for the best results, yet short enough to minimize toxicant-produced mortality.

## RESULTS

### Oxygen consumption rate of control group

The oxygen consumption rate of the 4 river puffer fish not exposed to PCP varied from ca 0.23 to 1.82 ml

$O_2$  (g dry wt) $^{-1}$  h $^{-1}$  with the mean consumption rate being  $0.82 \pm 0.01$  ( $x \pm SE$ ) ml  $O_2$  (g dry wt) $^{-1}$  h $^{-1}$ . It showed a daily rhythm having 5 periods in about 120.3 h of experiment (Fig. 2). Peaks of oxygen consumption occurred at intervals of ca 24 h measured at around 08:00 h.

### Effects of PCP on oxygen consumption rate

**Exposure to 50 ppb PCP.** The instantaneous rate of oxygen consumption by river puffer fish exposed to 50 ppb PCP increased for the first 20 h and then maintained the same rate throughout the test (Fig. 3). The mean oxygen consumption rate after exposure was 1.34 ml  $O_2$  (g dry wt) $^{-1}$  h $^{-1}$  which is 32.7% greater than that before the exposure (Table 1).

**Exposure to 100 ppb PCP.** Instantaneous rate of oxygen consumption of fish after exposure to 100 ppb PCP was sharply increased and showed clear daily fluctuations (Fig. 4). The mean oxygen consumption rates before and after exposure to 100 ppb averaged over the entire range of oxygen saturations were 1.07 and 1.67 ml (g dry wt) $^{-1}$  h $^{-1}$ , respectively. This shows that the fish at 100 ppb PCP consumed about 56.1% more oxygen than the unexposed fish. Oxygen consumption rate of the fish at 100 ppb PCP was about

Fig. 2. *Takifugu obscurus*. Time series of the oxygen consumption rate of 4 river puffer fish not exposed to PCP (control group). Fish (mean total length  $5.9 \pm 0.1$  cm, 4.3 g dry wt) were kept at 14.6°C and at oxygen saturation levels between 86.3 and 94.9% (SE =  $\pm 0.04$ ). (O) Mean oxygen consumption rate during 90 s; arrows indicate peaks of oxygen consumption at around 08:00 h

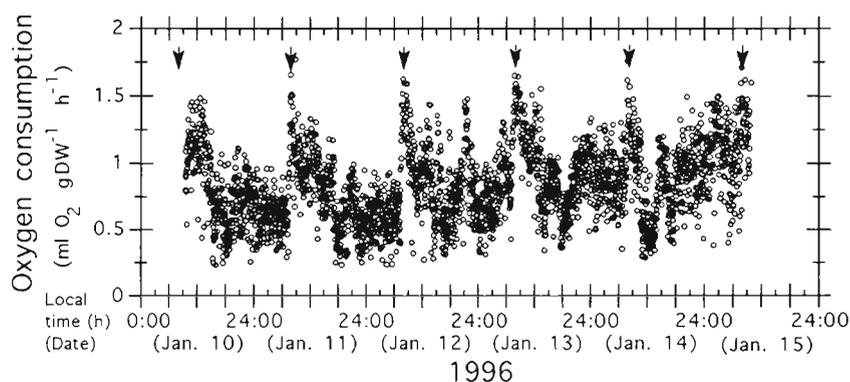


Fig. 3. *Takifugu obscurus*. Patterns of the oxygen consumption rate of 4 river puffer fish (mean total length  $6.1 \pm 0.1$  cm, 4.3 g dry wt) before and after exposure to 50 ppb PCP. Arrows indicate ~08:00 h

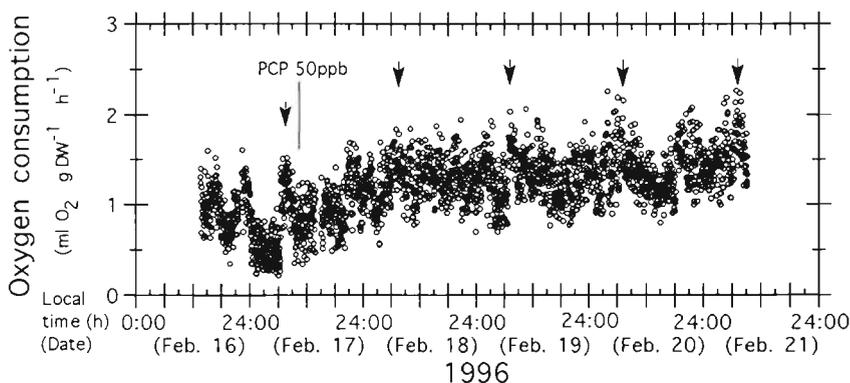


Table 1. *Takifugu obscurus*. Rates of oxygen consumption by the river puffer fish. Statistical values were computed for each batch from between 70 and 3185 measurements. Values are mean  $\pm$  standard error

	Control	50 ppb PCP	100 ppb PCP	200 ppb PCP	500 ppb PCP
Number of individuals (n)	4	4	4	4	4
Mean total length (cm)	5.9 $\pm$ 0.1	6.1 $\pm$ 0.1	6.2 $\pm$ 0.4	5.6 $\pm$ 0.2	6.4 $\pm$ 0.1
Total dry weight (g)	4.3	4.3	4.3	4.1	4.3
Salinity (‰)	30.1	30.8	31.1	31.1	30.3
Flow rate (ml min <sup>-1</sup> )	690	690	690	690	690
Temperature (°C)	14.6 $\pm$ 0.004	14.8 $\pm$ 0.004	14.7 $\pm$ 0.005	14.8 $\pm$ 0.004	14.6 $\pm$ 0.009
Level of oxygen saturation (%)	94.9–86.3 ( $\pm$ 0.04)	94.6–86.6 ( $\pm$ 0.04)	94.5–86.9 ( $\pm$ 0.06)	94.5–86.6 ( $\pm$ 0.05)	94.9–86.3 ( $\pm$ 0.04)
Number of points measured (after exposure)	3185	2048	886	1633	70
Duration (h) of the experiment after exposure to PCP	120.3	93.1	74.6	74.6	7.0
Measurement interval (s)	90	90	90	90	90
Mean oxygen consumption (ml O <sub>2</sub> g dry wt <sup>-1</sup> h <sup>-1</sup> )					
Before PCP exposure (A)	0.82 $\pm$ 0.01	1.01 $\pm$ 0.02	1.07 $\pm$ 0.01	1.26 $\pm$ 0.01	0.95 $\pm$ 0.02
After PCP exposure (B)	–	1.34 $\pm$ 0.01	1.67 $\pm$ 0.01	2.03 $\pm$ 0.01	1.58 $\pm$ 0.04
Increase in rate of oxygen consumption (%) = (B – A)/A	–	32.7	56.1	61.1	66.3

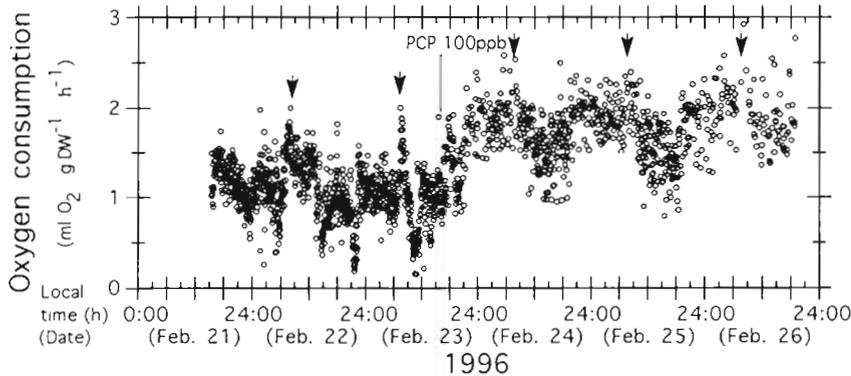


Fig. 4. *Takifugu obscurus*. Patterns of the oxygen consumption rate of 4 river puffer fish (mean total length 6.2  $\pm$  0.4 cm, 4.3 g dry wt) before and after exposure to 100 ppb PCP. Arrows indicate ~08:00 h

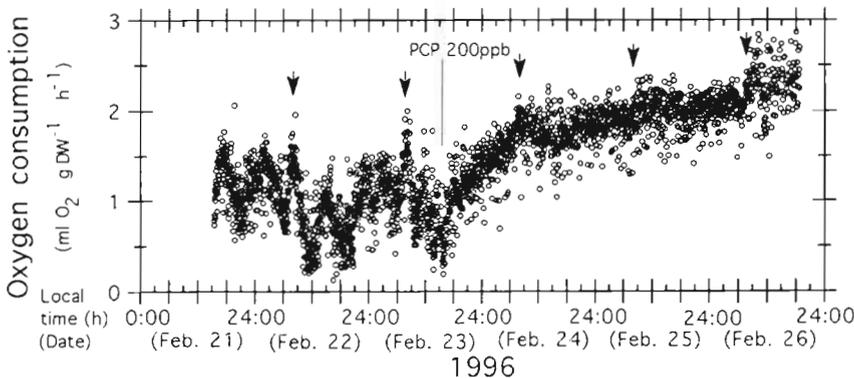


Fig. 5. *Takifugu obscurus*. Patterns of the oxygen consumption rate of 4 river puffer fish (mean total length 5.6  $\pm$  0.2 cm, 4.1 g dry wt) before and after exposed to 200 ppb PCP. Arrows indicate ~08:00 h

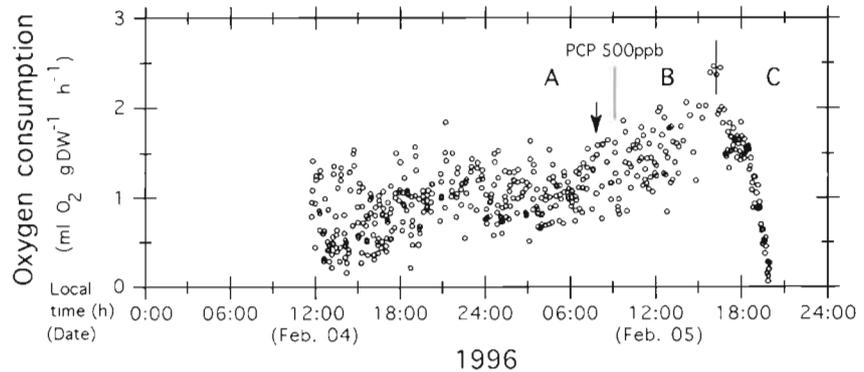
24.6% greater compared to fish at 50 ppb PCP (Table 1).

**Exposure to 200 ppb PCP.** The river puffer fish exposed to 200 ppb PCP exhibited a pattern of oxygen consumption that was quite different from those exposed to 50 and 100 ppb PCP. Both of the latter exhibited clear daily fluctuations (rhythms) of oxygen consumption (Figs. 3 & 4), whereas the former showed no such a pattern (Fig. 5). Mean oxygen consumption rate of fish at

200 ppb PCP increased to 2.03 ml O<sub>2</sub> (g dry wt)<sup>-1</sup> h<sup>-1</sup> which is 61.1% higher than that for fish before exposure.

**Exposure to 500 ppb PCP.** Prior to addition of PCP, the oxygen consumption rate was about 0.95 ml O<sub>2</sub> (g dry wt)<sup>-1</sup> h<sup>-1</sup> (Fig. 6, Period A). Following exposure to 500 ppb PCP, it increased to 1.58 ml O<sub>2</sub> (g dry wt)<sup>-1</sup> h<sup>-1</sup> (Fig. 6, Period B), about 66.3% higher than that for unexposed fish. All fish in the chamber died after 10 h exposure to 500 ppb PCP (Fig. 6, Period C).

Fig. 6. *Takifugu obscurus*. Patterns of the oxygen consumption rate of 4 river puffer fish (mean total length  $6.4 \pm 0.1$  cm, 4.3 g dry wt) before and after exposed to 500 ppb PCP. Arrow indicates ~08:00 h



## DISCUSSION

When fish are exposed to potential toxicants, the chemicals may directly affect metabolic reactions. Respiratory distress may arise as a result of either reduced oxygen diffusion over the gill membranes caused by an increase in the thickness of the mucous layer covering the secondary lamellae (Jobling 1994) or depleted haemoglobin content (Babu et al. 1985). Decrease in oxygen consumption rate with increasing time associated with the concentration of various pesticides in aquatic animals has been observed by a number of researchers (Babu et al. 1985, Muley & Mane 1987, Reddy et al. 1987, Ritakumari & Sreeletha 1987, Samb-siva-Rao et al. 1987, Mushithaq & Nagarajan 1992, Ramarkrishnan & Sivakumar 1993).

In river puffer fish, however, the oxygen consumption rate was increased when exposed to high concentrations of PCP up to 200 ppb. Even at 500 ppb PCP it increased by 66.3% in the present study. Similar results were found from a freshwater fish *Channa punctatus* by Gopal et al. (1989) and Sastry et al. (1991). The opposite results for the oxygen consumption rates of fish obtained in the previous studies and those of the present one cannot be immediately explained. This could be partly due to the differences in methodologies used. In the present study, oxygen consumption was measured by a sensitive AIFR which provided a constant oxygen tension at levels above 86% saturation in the test chamber throughout the experiment. Oxygen measurements in other studies using conventional methods were made in a closed system which could not keep a constant oxygen saturation level in the experimental chamber. A drop in oxygen tension below a certain level in the test chamber would lower the metabolic activities of organisms.

A daily rhythmicity of respiratory activity was maintained in concentrations of PCP up to 100 ppb, but was completely dampened when the fish were exposed to 200 and 500 ppb PCP. It appeared that concentrations of PCP over 200 ppb caused a strong physiological stress on the river puffer fish. Although the present

study was not designed to determine 24 to 72 h  $LC_{50}$  for PCP, it can be assumed from this experiment that the median lethal concentration for river puffer fish lies between 200 and 500 ppb PCP, since there was no mortality for the fish at 200 ppb, while all fish died within 10 h at 500 ppb.

Throughout the experiments, the *Takifugu obscurus* were kept away from any external stimuli such as light, food, temperature, salinity or tide which may have affected their rhythmic activity. A pilot study with unexposed fish exhibited the distinctive peaks of the oxygen consumption at around 08:00 h, which can be considered to be a circadian rhythm with a period of ca 24 h (Fig. 2). Fanta et al. (1990) also found that oxygen consumption rate of an Antarctic fish, *Notothernia neglecta*, showed 1 peak at 08:00 h. More work is needed to understand the observed daily rhythm of oxygen consumption and its relationships with intrinsic or extrinsic factors. The rhythmicity of oxygen consumption in river puffer fish observed in this study under constant conditions appears to have profound implications for their physiological processes, especially with regard to the respiratory energy cost, and, therefore, could be a good reference in determining the oxygen consumption rate of other fish.

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