Response of Larval and Postlarval Greasyback Prawns to Hypoxia

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Synopsis

Laboratory experiments were carried out using an oxygen and salinity gradient column to clarify the response to hypoxia of larval and postlarval greasyback prawns. The survival rate of the greasyback prawns increased in postlarval stage compared to mysis stage. They escaped from hypoxia at dissolved oxygen concentrations less than 2.1-2.6 $mgO_2\ l^{-1}$ for mysis larvae (10 days after hatching), less than 2.1-2.7 $mgO_2\ l^{-1}$ for P4 postlarvae (14 days after hatching) and less than 1.2-1.7 $mgO_2\ l^{-1}$ for P15 postlarvae (25 days after hatching). These results suggested that greasyback prawns strengthen their levels of tolerance to hypoxia in correspondence to the advancement of life stage from early postlarvae (P5) to late postlarvae (P15).

KEYWORDS: Hypoxia, Avoidance, Survival rate, Greasyback prawn, Larvae, Postlarvae, Oxygen-salinity gradient column

1. Introduction

Osaka Bay is well known as a rich sea where a total amount of 225 species of fisheries organisms, together with 15000- 28000 tons of annual fishing catch, have been recorded in recent years. A gradual restoration of dissolved oxygen (DO) has been observed in the bottom layer of the central and southern part of Osaka Bay, due mainly to a regulation of sewage discharge. However, concentrations of dissolved oxygen are still low, and below 3.6 $mgO_2\ l^{-1}$ at harbor areas of inner Osaka Bay in summer, which is thought to be a crucial level of dissolved oxygen required for the healthy growth of the marine benthic animals (Yanagi, 1989; Pihl et al., 1991; Kodama et al., 2006).

A committee of the Ministry of the Environment, Japan showed the importance of dissolved oxygen in the bottom water as an indicator of water quality, and examined its target values to maintain sound marine benthic biological resources. Yamochi et.al (1998) examined the tolerance to hypoxia of several benthic fish and crustaceans of Osaka Bay and demonstrated that dissolved oxygen should not drop below 1.6 $mlO_2\ l^{-1}$ (2.3 $mgO_2\ l^{-1}$) for longer than 1 day, and be kept higher than 2.6 $mlO_2\ l^{-1}$ (3.7 $mgO_2\ l^{-1}$) on average in summer to maintain sound populations of demersal fishes and crustaceans of the bay.

Greasyback prawn, *Metapenaeus ensis* is a major fishery species of Osaka Bay and known as a benthic crustacean highly enduring of hypoxic conditions (Yamochi et.al, 1995). Oda et al. (1997) analyzed the relationship between hypoxia and population dynamics of the juvenile greasyback prawn in Osaka Bay using a numerical model where they assumed all juveniles of the prawns escaped from hypoxia at a concentration of 2.1$mgO_2\ l^{-1}$. However, there is so far little information on the minimum dissolved oxygen required for the sound habitats for the whole life history of the greasyback prawns, including their larval stage. Therefore, we need more information on the physiological and/or ecological responses to hypoxia of the larval greasyback prawns, since the larval stage is expected to be less tolerant to severe oxygen conditions compared to adults of the same species.

The present paper attempts to clarify the change of tolerance or avoidance to hypoxia in correspondence to the growth of larval and postlarval greasyback prawns. For this purpose laboratory experiments were done to examine the survival or avoidance of hypoxia of the mysis and postlarval stage of the greasyback prawns using an oxygen and salinity gradient glass column.

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2. Methods

2.1 . Preparation of oxygen and salinity gradient column

Two liters of filtered seawaters (ca.30 in salinity) were concentrated or diluted to 40 and 20 salinity respectively, and added into two glass flasks. Seawater with high salinity was ventilated by N_2 gas to reduce the concentration of dissolved oxygen (Flask A in Figure1), while those with low salinity were aerated to adjust its concentration (Flask B in Figure1). The two flasks were connected to each other with Tygon tubing and the seawater in each flask gently stirred, then poured into a glass column of 86cm in height and 5cm in diameter using a roller pump (Furue Science Co., model RP-NB) at a flow rate of 120 ml min^{-1} (Tanaka, 1991). Prior to the addition of seawater to the glass column, the concentration of dissolved oxygen was checked with an Oxygurd DO meter (Type Handy Polaris). We referred to a glass column with salinity gradient but with no oxygen gradient as the control column where no ventilation of N_2 gas was made, and only salinity gradient was formed in the column.

2.2. Test organism

Mysis and postlarvae of the greasyback prawns, which were hatched and grown at the Mie Prefectural Sea Farming Center, were transferred to the laboratory and employed for the experiment (Figure 2). All larval and postlarval greasyback prawns were exposed to the seawater for ca.3 hours with gentle aeration before the experiment. Averaged body length was ca.2 mm for mysis (10 days after hatching), ca.3 mm for P4-stage postlavae (14 days after hatching) and ca.13 mm for P15-stage postlavae (25 days after hatching).

2.3. Observation

Pre-observation was carried out to confirm whether the oxygen and salinity gradient was maintained for 24 hours in a condition without the larvae of greasyback prawns. Dissolved oxygen was monitored at intervals of 3 hours for 24 hours at every 10cm depth of the oxygen and salinity gradient column with an Oxygurd DO meter. Density was checked at a water temperature of 25 °C using 4 standard density floats of 1.0150, 1.0175, 1.0200 and 1.0225 g cm^{-3} (Shibayama Scientific Co. LTD.), and was converted into salinity according to the Manual on Oceanographic Observation (Japan Meteorological Agency, 1999). As shown in Table 1, the oxygen gradient was kept for 24 hours when no larvae or postlarvae of greasyback prawn were added to the column except for lower layers at 6 hours. No clear difference was found in the position of each density float in the column before and after the experiment, which meant that salinity gradient was almost constant and didn't change for 24 hours. All of the seawaters used for the experiment were sterilized by autoclaving at 121°C for 5 min prior to the experiment.

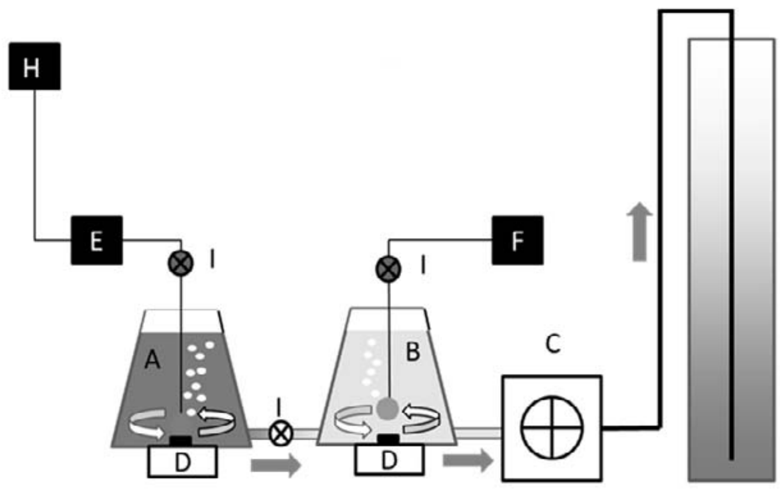

Fig.1: Schematic diagram of the experimental apparatus.
A: condensed seawater, B: diluted seawater, C: roller pump, D: stirrer, E: flow meter, F: air pump, G: oxygen and salinity gradient glass column, H:N_2 gas, I: stopper valve

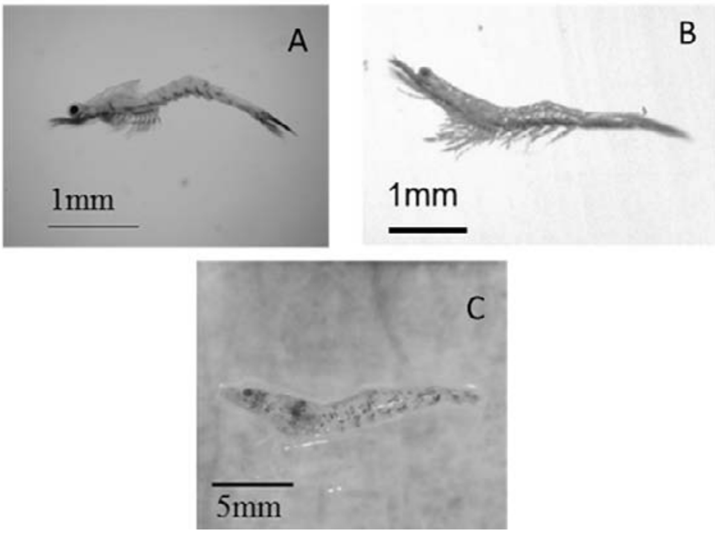

Fig. 2 : Mysis and postlarva of the greasyback prawn
A: Mysis (10 days after hatching), B:P4 stage postlarva (14 days after hatching), C:P15 stage postlarva (25 days after hatching)

Table 1 : Changes of dissolved oxygen concentration in the oxygen and salinity gradient column.

Depth	Time (h)								
(cm)	0	3	6	9	12	15	18	21	24
0	6.3	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
10	6.5	6.7	6.0	6.7	6.7	6.7	6.7	6.7	6.7
20	5.4	5.4	5.3	5.4	5.4	5.4	5.4	5.4	5.4
30	4.7	4.8	4.5	4.8	4.8	4.8	4.8	4.8	4.8
40	4.3	4.1	4.0	4.1	4.1	4.1	4.1	4.1	4.1
50	3.9	3.8	3.4	3.8	3.8	3.8	3.8	3.8	3.8
60	3.5	3.4	2.8	3.4	3.4	3.4	3.4	3.4	3.4
70	3.1	3.2	1.9	3.2	3.2	3.2	3.2	3.2	3.2
Bottom	2.9	3.0	1.7	3.0	3.0	3.0	3.0	3.0	3.0

* Value is shown in $mgO_2\ l^{-1}$.

Fifteen individuals of mysis, P4 and P15-stage larvae of greasyback prawn were added to the surface layer of the seawater (0-10 cm depth from the surface) on 0.5 hour before the onset of observation. Each glass column was kept at 25±1 °C with a photoperiod of 12 hours light and 12 hours dark at a photon density of 90-100 μmol m^{-2} sec. We observed the number of individuals of the larval or postlarval greasyback prawn at every 1cm depth from the surface to the bottom of the column at 3 hours intervals and compared with those of the control one. An overall percentage of distribution of the larval or postlarval greasyback prawns was calculated by averaging the percentage of distribution of each observation time at layers of 0-30 cm, 30-60 cm and 60 cm-bottom, respectively. Vertical distributions of dissolved oxygen were checked at 8 hours intervals to confirm the oxygen gradient to be maintained.

3. Results

3.1. Survival rate

Survival rates of the larval and postlarval greasyback prawns in the oxygen and salinity gradient column were shown in Table 2. We regarded larval and postlarval greasyback prawns as dead in the following case:
(1) They lost their mobility and showed no response when touched with a needle in the fresh seawaters.
(2) Their body color has changed from yellowish brown to pink.

In addition, data from 0 to 15 hours were used since dissolved oxygen concentrations remarkably decreased owing to the effects of decomposition of dead prawns at the bottom of the column after 16 hours. Survival rate of the mysis-stage larvae decreased to 60.0% when dissolved oxygen concentrations dropped to 0.9-1.6 $mgO_2\ l^{-1}$at the bottom of the column (case 4), while the rate was kept at a range of 86.7 to 100% at 1.8-2.0 $mgO_2\ l^{-1}$ (case 3), 2.3-2.6 $mgO_2\ l^{-1}$ (case 2), 2.8-3.3 $mgO_2\ l^{-1}$ (case 1) and 5.8-5.9 $mgO_2\ l^{-1}$ (control). The P4-stage postlarvae showed a high survival rate of 93.3% under dissolved oxygen concentrations of 2.0-2.3 $mgO_2\ l^{-1}$ (case 3), 2.1-2.7 $mgO_2\ l^{-1}$ (case 2), 2.7.2-3.1 $mgO_2\ l^{-1}$ (case 1) and 5.3-5.7 $mgO_2\ l^{-1}$ (control) but the survival rate remarkably decreased (53.3%) at 1.6-1.7 $mgO_2\ l^{-1}$ (case 4). In contrast, the P15-stage postlarvae endured hypoxic seawaters for 15 hours and showed high survival rates of 86.7-100% even when they encountered dissolved oxygen concentrations of 0.2-1.7 $mgO_2\ l^{-1}$ (case 4), 0.7-2.3 $mgO_2\ l^{-1}$ (case 3), 0.3-2.7 $mgO_2\ l^{-1}$ (case 2), 1.1-3.1 $mgO_2\ l^{-1}$ (case 1) and 1.5-5.7 $mgO_2\ l^{-1}$ (control) at the bottom of the column. No clear difference was observed in the tolerance of hypoxia between the mysis and P4 postlarval stage of the greasyback prawns.

Table 2: Survival rate of the larval and postlarval greasyback prawns.

Stage	Control	Oxygen - salinity gradient column			
		Case1	Case2	Case3	Case4
Mysis larvae	86.7	100	86.7	86.7	60.0
P4-stage postlarvae	93.3	93.3	93.3	93.3	53.3
P15-stage postlarvae	93.3	100	100	93.3	86.7

* Value is survival rate of 0-15 hours and shown in percentage.

**Initial dissolved oxygen concentaration at the bottom of the column,

Case1:3.1-3.3 $mgO_2\ l^{-1}$, Case2:2.6-2.7 $mgO_2\ l^{-1}$, Case3:2.0-2.3 $mgO_2\ l^{-1}$, Case4:1.6-1.7 $mgO_2\ l^{-1}$, Control:5.7-5.9 $mgO_2\ l^{-1}$

3.2. Avoidance

1) Mysis

Distributions of the mysis-stage larvae of the greasyback prawns in the oxygen and salinity gradient column were shown in Table 3. Avoidance of the larvae to hypoxia was analyzed based on the results of 0-15 hours of the time course, since dissolved oxygen decreased at the upper or middle layer of the column between 16 and 24 hours. When dissolved oxygen at the bottom of the column at 0-16 hours was 2.8-3.3 $mgO_2\ l^{-1}$(case 1), a 71% of the live larvae distributed at a layer of 0-60 cm from the water surface. In cases 2 and 3 (dissolved oxygen at the bottom of the column at 0-16 hours was 2.3-2.6 $mgO_2\ l^{-1}$ for case 2 and 1.8-2.0 $mgO_2\ l^{-1}$ for case 3), a total of 84.6% and 94.0% of the mysis-stage larvae were observed at the 0-60 cm layer. A marked decline of individuals of the surviving larvae was found from 15 to 18 hours in case 4, resulting in only a few larvae remained at the end of the experiment. At 0-15 hours of the case 4 (dissolved oxygen at the bottom: 0.9-1.6 $mgO_2\ l^{-1}$), the percentage of distribution of the live mysis larvae attained 91.2% at the 0-60 cm layer.

2) P4-stage postlarva

Distributions of the P4-stage postlarvae of the greasyback prawns in the oxygen and salinity gradient column were depicted in Table 4. As dissolved oxygen decreased in the column after 16 hours as with the mysis-stage experiment, avoidance of the P4 postlarvae to hypoxia was analyzed based on the results of 0-15 hours. In the control column, the percentages of distribution of the postlarvae were ca. 60% at the bottom of the column, while those in the oxygen and salinity gradient column were low and showed 46.6% at dissolved oxygen concentrations of 2.7-3.1 $mgO_2\ l^{-1}$ in case 1, 37.5% at 2.4-2.7 $mgO_2\ l^{-1}$ in case 2, 25.6% at 2.0-2.3 $mgO_2\ l^{-1}$ in case 3 and 18.8% at 1.6-1.7 $mgO_2\ l^{-1}$ in case 4. As a result, the percentage of distribution of the P4-stage postlarvae at the bottom of the column clearly decreased with decreasing dissolved oxygen. In contrast, they were well observed at a layer of 30-60 cm from the surface in each oxygen and salinity gradient column where dissolved oxygen concentration ranged between 3.2-5.2 $mgO_2\ l^{-1}$ (case 1), 3.6-4.7 $mgO_2\ l^{-1}$ (case 2), 3.6-4.4 $mgO_2\ l^{-1}$ (case 3),and 2.8-4.7 $mgO_2\ l^{-1}$ (case 4).

3) P15-stage postlarva

Table 5 showed distributions of the P15-stage postlarvae of the greasyback prawns in the oxygen and

Table 3 : Distributions of the mysis-stage larvae of the greasyback prawns.

	Depth (cm)	DO concentration (mg l^{-1}) start	8h	16h	24h	Salinity start	Number of individuals start	3h	6h	9h	12h	15h	18h	21h	24
Control	surface -10	6.6	6.6	6.6	5.5	21.7	2	0	2	1	0	1	4	3	2
	10-20	6.1			5.0	23.5	0	1	1	0	1	0	1	0	2
	20-30	6.4		5.8	4.4	25.3	2	2	1	4	3	2	2	2	2
	30-40	6.1			4.3	27.1	3	2	7	4	0	2	3	3	1
	40-50	6.1	5.9	5.9	5.6	28.9	2	3	1	3	3	3	1	2	2
	50-60	6.0			5.6	30.7	1	0	0	0	4	4	0	1	0
	60-70	6.0		5.9	5.9	32.5	3	0	0	1	2	1	0	0	0
	70-bottom	6.0			5.7	34.2	0	4	0	0	0	0	1	1	1
	bottom	5.9	5.5	5.8	4.7	36.0	2	2	1	0	0	0	0	0	0
Case1	surface -10	6.6	6.6	6.3	5.1	21.6	1	0	1	0	1	1	1	4	3
	10-20	6.2		5.1	3.5	23.6	1	1	1	3	1	2	1	1	4
	20-30	5.7	5.4	4.9	3.6	25.4	1	2	0	2	2	3	2	4	4
	30-40	5.1		4.7	3.9	27.3	1	2	2	3	2	1	7	4	1
	40-50	4.8	4.3	4.5	3.7	29.1	4	3	1	1	1	0	2	1	1
	50-60	4.3		3.9	3.9	31.0	3	2	4	5	3	3	0	0	0
	60-70	3.8	3.8	3.6	3.8	32.9	2	2	3	1	2	4	1	0	0
	70-bottom	3.4		3.3	3.7	34.7	1	1	0	0	2	1	0	0	0
	bottom	3.3	3.0	2.8	3.2	36.6	1	2	3	0	1	0	0	0	0
Case2	surface -10	6.5	6.4	5.8	4.7	22.6	1	3	4	1	0	0	7	5	3
	10-20	5.8			3.1	24.3	4	2	1	0	1	1	0	2	2
	20-30	5.0	5.0	4.4	3.3	26.1	2	3	1	1	1	3	3	0	1
	30-40	4.7			3.4	27.8	2	3	3	2	3	2	2	3	1
	40-50	4.2	3.9	4.1	3.3	29.5	3	2	0	5	2	0	1	1	0
	50-60	3.9			3.4	31.2	1	1	2	3	5	4	0	0	0
	60-70	3.3	3.3	3.3	3.3	32.9	0	0	2	2	1	1	0	1	0
	70-bottom	2.9			3.0	34.6	0	0	0	0	0	0	0	0	0
	bottom	2.6	2.5	2.3	2.5	36.3	2	1	1	1	0	2	0	0	1
Case3	surface -10	6.7	6.3	6.3	4.5	22.4	2	3	1	2	1	1	7	5	4
	10-20	5.6			4.1	24.3	4	3	0	2	1	1	1	1	2
	20-30	4.8	4.7	4.1	2.7	26.2	3	2	3	2	1	2	2	4	2
	30-40	4.1			2.5	28.2	4	2	2	3	7	0	2	2	2
	40-50	3.5	3.7	3.5	2.5	30.1	2	3	2	4	3	6	0	1	1
	50-60	3.0			2.5	32.1	0	2	1	0	0	3	1	0	0
	60-70	2.6	2.6	2.6	2.4	34.0	0	0	1	0	0	0	0	0	0
	70-bottom	2.1			2.4	35.8	0	0	2	0	0	0	0	0	0
	bottom	2.0	1.8	1.8	1.4	37.7	0	0	2	0	0	0	0	0	0
Case4	surface -10	6.4	6.3	5.9	4.6	23.0	0	2	1	1	1	0	1	1	1
	10-20	5.7		4.9	3.4	24.7	2	2	0	0	0	1	0	0	1
	20-30	4.8	4.5	4.5	2.9	26.4	5	2	5	2	2	2	1	1	0
	30-40	4.2		4.2	2.6	28.1	1	5	3	2	2	3	0	0	0
	40-50	3.5	3.2	3.6	2.4	29.8	4	0	1	4	3	2	1	0	0
	50-60	2.8		2.8	2.4	31.5	0	1	1	0	0	1	0	1	0
	60-70	2.2	2.3	2.0	2.0	33.1	0	0	0	0	0	0	0	0	0
	70-bottom	1.9		1.5	1.4	34.8	0	0	0	1	1	0	0	0	0
	bottom	1.6	1.3	0.9	0.7	36.5	2	2	0	0	0	0	0	0	0

: showed dark period

Table 4：Distributions of the P4-stage postlarvae of the greasyback prawns.

	Depth	DO concentration (mg l^{-1})				Salinity	Number of individuals								
	(cm)	start	8h	16h	24h	start	start	3h	6h	9h	12h	15h	18h	21h	24h
Control	surface -10	6.6		6.3	5.9	22.1	0	0	0	0	0	0	0	0	0
	10-20	6.5			5.1	23.8	0	0	0	0	0	1	0	0	0
	20-30	6.2		5.7	5.1	25.5	0	0	0	0	0	1	0	0	0
	30-40	6.4			5.5	27.3	0	0	1	0	2	0	0	0	0
	40-50	6.1		5.7	5.5	29.0	0	2	1	3	4	1	2	0	0
	50-60	6.0			5.3	30.7	0	0	4	4	0	1	2	0	0
	60-70	5.8		5.6	5.4	32.4	0	0	2	2	2	1	3	0	1
	70-bottom	5.8			5.2	34.1	1	0	1	1	1	0	2	1	1
	bottom	5.7		5.3	4.0	35.8	14	13	6	5	6	9	4	12	9
Case1	surface -10	6.6	6.2	5.7	4.8	21.2	0	0	0	0	0	0	0	0	0
	10-20	6.1		4.8	3.6	23.2	1	1	0	0	0	0	0	2	0
	20-30	5.6	5.4	4.1	3.0	25.3	1	2	1	5	1	0	5	2	2
	30-40	5.2		3.6	3.0	27.3	0	1	3	6	0	4	1	3	2
	40-50	4.5	4.5	3.6	2.3	29.3	0	1	0	0	1	1	1	1	1
	50-60	4.2		3.2	2.4	31.3	0	0	1	0	2	1	1	1	0
	60-70	3.6	3.6	3.5	2.1	33.3	1	0	3	1	2	1	1	0	0
	70-bottom	3.1		2.8	2.2	35.3	0	0	3	0	3	0	2	1	1
	bottom	3.1	3.1	2.7	1.2	37.4	12	10	4	3	5	7	1	1	0
Case2	surface -10	6.3	5.9	6.3	5.4	21.9	0	1	0	0	0	0	0	0	0
	10-20	5.5			4.5	23.7	1	0	1	2	1	1	0	0	1
	20-30	5.1	4.4	4.8	3.6	25.6	0	1	4	3	0	3	4	0	1
	30-40	4.7			3.2	27.4	0	1	4	2	3	0	2	2	0
	40-50	4.0	3.7	4.2	2.7	29.2	2	3	1	1	2	1	1	1	2
	50-60	3.6			2.6	31.1	0	1	1	1	1	3	1	2	2
	60-70	3.2	3.1	3.2	2.4	32.9	2	1	0	1	1	2	0	0	0
	70-bottom	3.0			2.1	34.7	0	1	0	0	1	1	0	2	0
	bottom	2.7	2.1	2.4	1.9	36.5	10	6	4	5	5	3	2	2	1
Case3	surface -10	6.4	6.4	5.7	5.5	21.9	0	0	1	0	0	0	1	1	0
	10-20	5.6			4.2	23.7	0	1	3	0	2	0	0	1	0
	20-30	5.3	5.2	4.4	3.8	25.5	1	3	0	0	2	3	0	2	1
	30-40	4.4			3.4	27.3	1	1	2	2	2	3	3	0	0
	40-50	4.0	3.8	3.8	2.7	29.0	1	1	2	3	1	6	0	2	2
	50-60	3.6			2.4	30.8	2	2	1	2	1	0	0	2	3
	60-70	2.9	2.9	2.6	2.5	32.6	1	1	2	1	1	1	2	0	1
	70-bottom	2.5			1.9	34.4	1	1	1	2	2	1	1	1	2
	bottom	2.3	2.4	2.0	1.5	36.1	8	5	2	4	3	0	5	3	1
Case4	surface -10	6.1	6.2	5.6	5.5	21.6	1	1	1	0	0	0	2	0	0
	10-20	5.5		4.9	3.9	23.5	1	0	0	1	0	1	0	0	0
	20-30	5.2	4.9	4.3	3.6	25.4	1	2	3	1	2	1	1	2	2
	30-40	4.7		3.8	3.3	27.2	1	2	3	2	4	1	2	0	1
	40-50	4.0	4.2	3.4	2.7	29.1	2	2	2	5	2	3	0	2	1
	50-60	3.3		2.8	2.3	30.9	1	1	1	1	1	1	2	0	0
	60-70	2.5	2.4	2.0	1.6	32.8	1	1	0	0	1	1	0	0	0
	70-bottom	2.0		1.8	1.4	34.6	0	1	0	0	0	0	0	0	0
	bottom	1.7	1.7	1.6	0.8	36.5	7	2	2	2	0	0	0	0	0

■ : showed dark period

Table 5 : Distributions of the P15-stage postlarvae of the greasyback prawns.

	Depth	DO concentration (mg l^{-1})				Salinity	Number of individuals								
	(cm)	start	8h	16h	24h	start	start	3h	6h	9h	12h	15h	18h	21h	24h
Control	surface -10	6.6	6.2	5.7	5.1	22.1	0	1	0	3	0	0	0	0	0
	10-20	6.5	6.1	5.0	4.4	23.8	2	2	0	2	0	0	0	0	0
	20-30	6.2	5.8	4.8	3.8	25.5	0	2	0	0	2	1	0	1	0
	30-40	6.4	5.7	5.1	3.7	27.3	0	1	2	0	2	0	1	1	1
	40-50	6.1	5.8	5.2	3.7	29.0	0	2	2	1	0	0	0	0	1
	50-60	6.0	5.8	5.4	4.4	30.7	0	0	0	0	0	0	0	1	4
	60-70	5.8	5.8	5.5	1.6	32.4	0	0	3	0	1	1	1	3	7
	70-bottom	5.8	5.8	2.1	1.3	34.1	0	0	0	0	2	1	2	2	1
	bottom	5.7	5.6	1.5	1.1	35.8	13	7	8	8	7	11	10	6	0
Case1	surface -10	6.6	5.5	4.6	4.2	21.2	0	1	0	1	0	0	0	0	1
	10-20	6.1	5.1	4.3	2.6	23.2	0	1	1	0	0	0	0	0	3
	20-30	5.6	4.9	3.8	2.1	25.3	0	1	1	0	1	1	1	2	3
	30-40	5.2	4.7	3.5	1.5	27.3	2	1	0	1	2	4	4	5	6
	40-50	4.5	4.0	3.3	1.1	29.3	1	2	0	1	3	2	1	5	2
	50-60	4.2	3.5	3.1	0.9	31.3	0	0	2	1	2	0	3	3	0
	60-70	3.6	2.9	2.1	0.7	33.3	2	1	0	2	3	0	4	0	0
	70-bottom	3.1	2.2	1.3	0.4	35.3	2	1	0	3	1	2	2	0	0
	bottom	3.1	1.2	1.1	0.3	37.4	8	7	11	6	3	6	0	0	0
Case2	surface -10	6.3	5.3	5.2	4.8	21.9	1	4	1	2	0	0	0	0	1
	10-20	5.5	5.1	5.0	4.5	23.7	0	1	0	1	0	1	0	1	1
	20-30	5.1	4.9	4.4	4.3	25.6	0	1	1	1	1	0	2	0	3
	30-40	4.7	4.6	4.1	3.5	27.4	0	0	1	0	0	2	0	1	0
	40-50	4.0	4.5	3.3	2.8	29.2	0	0	1	2	3	0	0	1	0
	50-60	3.6	3.5	2.3	2.4	31.1	0	1	3	3	1	2	0	2	0
	60-70	3.2	3.2	1.7	0.5	32.9	0	4	2	1	0	0	3	0	0
	70-bottom	3.0	1.8	1.2	0.5	34.7	2	0	3	2	0	0	0	0	0
	bottom	2.7	1.3	0.3	0.1	36.5	12	4	3	3	10	0	0	0	0
Case3	surface -10	6.4	5.2	5.2	5.4	21.9	0	3	1	0	0	0	0	1	1
	10-20	5.6	5.0	4.7	4.8	23.7	0	3	1	0	0	1	0	1	1
	20-30	5.3	4.8	4.1	3.6	25.5	0	1	1	1	0	5	0	2	4
	30-40	4.4	4.4	3.7	2.6	27.3	0	0	1	1	3	0	2	0	3
	40-50	4.0	4.3	3.2	1.8	29.0	0	0	0	0	1	1	1	2	4
	50-60	3.6	3.7	2.6	1.3	30.8	1	0	0	2	2	2	6	2	0
	60-70	2.9	2.8	1.6	1.1	32.6	0	0	3	1	3	3	3	0	0
	70-bottom	2.5	1.9	1.2	0.7	34.4	1	0	1	0	0	1	1	1	0
	bottom	2.3	1.3	0.7	0.1	36.1	13	8	7	10	5	1	0	4	0
Case4	surface -10	6.1	5.3	5.2	5.2	21.6	2	2	0	0	0	0	0	0	2
	10-20	5.5	5.1	4.7	4.3	23.5	2	0	0	0	0	0	0	0	0
	20-30	5.2	4.9	3.9	1.8	25.4	0	5	0	1	0	0	2	7	6
	30-40	4.7	4.5	3.0	1.4	27.2	1	1	0	1	1	0	3	0	4
	40-50	4.0	3.6	2.2	1.1	29.1	2	1	1	1	3	4	2	2	0
	50-60	3.3	3.1	1.5	0.6	30.9	1	0	7	7	5	5	3	2	0
	60-70	2.5	2.1	1.2	0.2	32.8	0	0	0	2	2	0	0	0	0
	70-bottom	2.0	1.8	0.7	0.1	34.6	2	1	4	1	0	2	0	1	0
	bottom	1.7	1.2	0.2	0.1	36.5	5	4	2	1	2	2	3	0	0

■ : showed dark period

salinity gradient column. As dissolved oxygen concentrations largely decreased by 16 hours in all oxygen and salinity gradient columns together with the control one, we used data of 0 to 8 hours for analyzing the avoidance to hypoxia of the P15-stage postlarvae. The percentage of distribution at the bottom of the column showed 57.8% in case 1 (1.2-3.1 $mgO_2\,l^{-1}$), 42.2% in case 2 (1.3-2.7 $mgO_2\,l^{-1}$) and 62.2% in case 3 (1.3-2.3 $mgO_2\,l^{-1}$), which had no remarkable difference with the value of 62.2% of the control column (5.6-5.7 $mgO_2\,l^{-1}$). However, only 2-5 individuals of the P15 postlarvae were observed at the bottom when dissolved oxygen decreased between 1.2 and 1.7 $mgO_2\,l^{-1}$ in case 4. Although dissolved oxygen strikingly declined after 8 hours, P15-stage postlarvae survived in the latter half of the experiment except for case 2 where 10 individuals became inactive at 12 hours and died thereafter.

Table 6 : Criteria of avoidance to hypoxia for the mysis or postlarvae of the greasyback prawns.

Stage	DO concentration ($mgO_2 l^{-1}$)
Mysis-stage larvae	2.1-2.6
P4-stage postlarvae	2.1-2.7
P15-stage postlarvae	1.2-1.7

4. Discussion

Miller et al. (2002) examined the hypoxic tolerance of 13 species of marine and estuarine fishes, crustaceans and a bivalve and reported that mortality attained 14-22% for crustaceans even in the control test due to their metamorphosis and molting. They also suggested that the responses to hypoxia can be compared to each other if the mortality in the control is below these percentages. In the present experiment mysis-stage greasyback prawns showed a mortality of 13.3% in the control column which is lower than a range described by Miller et al. (2002). Yamochi et al. (1995) carried out indoor experiments and field surveys on the hypoxic tolerance of the juvenile greasyback prawn, indicating that they can survive for 24 hours at dissolved oxygen saturations of 20-27% (1.4-1.9 $mgO_2 l^{-1}$) with LC-50 being less than 7-12% (0.50-0.87 $mgO_2 l^{-1}$) at 25 °C. And the juvenile greasyback prawn can survive at low concentrations of dissolved oxygen compared with juvenile kuruma prawn *Marsupenaeus japonicus*, young swimming crab *Portunus trituberculatus*, and young common brackish goby *Acanthogobius flavimanus*. The juvenile greasyback prawn's habitat was at the bottom of the mouth of the Yodo river, Osaka, where oxygen saturation decreased to 12-55% in summer. These findings lead to a conclusion that the greasyback prawns can dominate in hypoxic habitats depending on their excellent ability of tolerance to hypoxic conditions. In the present study, P15 postlarvae of the greasyback prawns, whose body length were ca.15 mm survived at a rate of 86.7% for 15 hours and 80.0% for 24 hours at highly hypoxic conditions of 0.1-3.1 $mgO_2 l^{-1}$ (Tables 2 and 4). This well coincided with occurrence of the juvenile greasyback prawns with 25-40 mm body length in the hypoxic estuary of the Yodo river. Although the mortality sharply increased at 0.7-1.6 $mgO_2 l^{-1}$ for mysis and at 0.8-1.7 $mgO_2 l^{-1}$ for P4 postlarvae, no clear increase in mortality was observed at 0.1-1.7 $mgO_2 l^{-1}$ for P15 postlarvae. This suggests that the greasyback prawns may acquire hypoxic tolerance between P4 and P15 postlarval stage, and they then succeed in occupying their habitats in hypoxic coastal areas or river mouths owing to their prominent tolerance to low oxygen.

Statistical analysis (χ^2 test) was used to determine the concentration of dissolved oxygen where the larvae or postlarvae started avoiding. In this case, we employed the experimental data of 0-15 hours which had no large decline of the concentrations of dissolved oxygen. A statistical difference was detected in the vertical distribution of the mysis-stage larvae between case 3 (dissolved oxygen concentration at the bottom:1.8-2.0 $mgO_2 l^{-1}$) and control. In addition, there was a statistical difference of the distribution between case 3 and control even when data of the 70cm-bottom layer were excluded ($P < 0.10$). Therefore, the upper limit of avoidance against hypoxia in the mysis-stage larvae was estimated to be 2.1-2.6 $mgO_2 l^{-1}$. Vertical distributions of the P4-stage postlarvae were different in case 2, case 3 and case 4 if compared to the control ($P < 0.05$). This suggests that the upper limit of avoidance in P4-stage larvae was between 2.1-2.7 $mgO_2 l^{-1}$ (dissolved oxygen concentrations at the bottom of the column in case 2). After growing into P15-stage larvae, the statistical difference of vertical distribution ($P < 0.05$) was only confirmed between case 4 (dissolved oxygen concentration at the bottom: 1.2-1.7 $mgO_2 l^{-1}$) and control, but not between case 1, case 2, case 3 and control when compared the number of individuals of the larvae at the bottom. This indicates that P15-stage larvae of the greasyback prawns start avoidance to hypoxia at 1.2-1.7 $mgO_2 l^{-1}$. As a conclusion, the larvae of the greasyback prawns avoid hypoxia at dissolved oxygen concentrations below 2.1-2.6 $mgO_2 l^{-1}$ for mysis larvae, below 2.1-2.7 $mgO_2 l^{-1}$ for P4 postlarvae and below 1.2-－1.7 $mgO_2 l^{-1}$for P15 postlarvae (Table 6). These results reveal that physiological limit of avoidance to hypoxia of the larval greasyback prawns becomes low in correspondence to the development of life stage, especially from early postlarvae to late postlarvae. This also corresponds with the findings that survival rate of the greasyback prawns increase depending on the stage from early postlarvae (P4) to late postlarvae (P15).

Ohtomi et al.(2006) reported that the rate of settlement of the larval mantis shrimp *Oratosquilla oratoria* decreased depending on the hypoxic conditions in Tokyo Bay. Further, Kusakabe et al.(2002) demonstrated that hypoxic bottom water could affect the distribution of juvenile southern rough shrimp *Trachysalambria curvirostris* in August in Osaka Bay. These results lead to a conclusion that the juvenile greasyback prawns can dominate in hypoxic habitats of inner Osaka Bay owing to their excellent ability of tolerance to hypoxic conditions, but spatial distribution of the greasyback prawns changed and their growth was inhibited by hypoxia when they were in mysis or early postlarval stage.

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