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Prey Survival and Vulnerability of Juvenile Rhynchocypris oxycephalus in Juvenile Fish Shelters under Predation by Korean Native Piscivorous Fish (Coreoperca herzi)

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Abstract: The aim of this study was to evaluate the newly-developed juvenile fish shelter (JFS) for its ability to increase prey survival and to improve species diversity in a freshwater ecosystem. An experiment was performed in an outdoor large-scale mesocosm three times from 2011 to 2012 by comparing the responses to adjustment as a function of the volume of JFS in the control and experimental groups. Analysis results of the environmental monitoring over three periods indicated only minor differences in the physicochemical characteristics of the water quality and phyto- and zoo-plankton biomass, thereby enabling a comparative analysis of the feeding ecology. However, the water temperature exhibited large fluctuations, ranging from 16.4 to 27.6 °C, and high water temperature conditions (Period 1, 25.6 \pm 2.0 °C) enhanced the predation activity of the piscivorous fish *Coreoperca herzi* (*C. herzi*, size 89 \pm 4 mm). Statistically, the survival rates of the prey fish, *Rhynchocypris oxycephalus* (*R. oxycephalus*, size 29 \pm 1 mm), with JFSs were greater by 35.9%–46.7%, and improved as the patch volume of JFS increased. Based on both experimental observations and statistical analysis, the JFS developed in this study could reduce the chances of predator–prey encounters, minimize prey vulnerability, and thereby increase prey survival rates.

Keywords: *C. herzi*; juvenile fish shelter (JFS); piscivorous fish; prey fish; *R. oxycephalus*; survival rates; vulnerability

1. Introduction

Fish are generally considered as a major component of the food chain, and maintain the balance of aquatic ecosystems [1]. As a valuable aquatic resource, juvenile fish are important to improve biodiversity and to maintain a healthy aquaculture environment [2]. However, most juvenile fish encounter various predators during their growth. The stage at which juvenile fish grow into adult fish is an important period of their life since most juvenile fish are easily preyed upon during the early stage of their life [3,4].

Due to this vulnerability to predation of juvenile fish, juvenile fish have acquired various behaviors to avoid predators: (1) switching habitats; (2) reducing exposure time; (3) increasing vigilance; and (4) shifting activity times. To minimize predator encounters, most juvenile fish switch habitats to the edges of the water body [3,4]. However, due to the lack of food and the presence of the inter-species competition in such densely populated habitats, juvenile fish often exhibit retarded growth rates [4].

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In addition, starving individuals may forage in open waters, but most of these juvenile fish are eaten by predators [5–7].

Although population balance among different species is generally conserved by complex feeding predator–prey relationships in natural habitats, feeding relationships within simple food chains in artificial habitats (e.g., artificial rivers and lakes) can make certain species extinct [8]. And, high levels of exploitation by both commercial and recreational fisheries over the world make many juvenile fish species under continuous survival pressure, demanding that cautious approaches to protect various juvenile fish species are required for future sustainability. Furthermore, exotic species such as bluegills and bass were reported to disturb the ecosystem and to make many native fish species extinct in Republic of Korea [9,10]. Thus, the protection of native juvenile fish is extremely important to sustain the ecosystem services, considering juvenile fish serve as passive links between aquatic ecosystem and among other food webs [8]. Since both survival rates and abundances of juvenile fish are critical factors influencing the variability in abundances of adult fish in rivers and lakes with environmental changes, there is a need to provide suitable habitats for both survival and protection of juvenile fish for future sustainability [11,12].

According to previous studies, various habitats are utilized by aquatic organisms [13,14]. For example, the use of river bed materials [15,16], plants in the littoral zone [17], fish reefs [18,19], and floating boxes [20] has been observed, and these habitats have been evaluated with respect to enhancement of survival rates for fish (especially juvenile) and/or crustaceans [21]. Moreover, an increase in the structural complexity (e.g., dense plants, wood, shade, etc.) of these habitats positively affects predator avoidance [22]. Thus, juvenile fish prefer structurally-complex habitats [23,24]. However, structurally-complex habitats are not entirely safe because predators can occasionally enter these habitats or attack by ambush. Nevertheless, juvenile fish have been found to prefer various moving habitats on the edges of the water body [25], because structurally-complex habitats on the edges of the water body are more beneficial for survival than open waters.

In this study, rectangular cube [0.5 m (W) \times 0.5 m (L) \times 0.3 m (H)] juvenile fish shelter (JFS) was developed, and evaluated as a suitable habitat in large-scale mesocosm experiment on three occasions from 2011 to 2012. The specific objectives of this study were: (1) to investigate the feasibility of the developed JFS as a suitable habitat to improve the survival rates of native juvenile fish under the predation by piscivorous fish in a freshwater ecosystem; and (2) to quantitatively evaluate the performance of the developed JFS installed in a large-scale mesocosm experiment. The results of this study can be utilized to optimize several factors for the design of JFS and to improve the survival rates of juvenile fish in various aquatic ecosystems.

2. Materials and Methods

2.1. Installation of Experimental Equipment

A large-scale mesocosm experiment was performed at the River Experiment Center (REC) of the Korea Institute of Civil Engineering and Building Technology (KICT) in the Nakdong river basin of the Republic of Korea. The specifications of the large-scale mesocosm experiment are provided in Figure 1. An outdoor water channel [2.0 m (W) \times 5.0 m (L) \times 1.2 m (H)] made of concrete was divided into four sections where the experimental equipment was installed. The isolated experimental sections were covered with fine-mesh nylon nets (2 \times 2 mm) to minimize the external interference. Similar to the previous study [26], shading net with 30% light penetration was installed to make predator fish feed more actively based on the nocturnal activities of piscivorous fish. In addition, the installed shading net prevents the predation of fish from mammalian and avian predators. The newly-developed JFSs were installed with different numbers: Control (no JFS), T1 (one JFS), T2 (two JFSs), and T3 (four JFSs).

As shown in Figure 2, each JFS was a rectangular cube that was hollow inside, and allowed juvenile fish to pass through the entrances [47 cm $(W) \times 1.5$ cm (H)] located on all sides of JFS. The installed JFSs were covered with fiber nets to increase the periphyton biomass for prey's food

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source. Six-week large-scale mesocosm experiment with different numbers of JFSs was performed three times (Periods 1–3) between August 2011 and May 2012 (see Table 1).



Figure 1. Pictorial view of large-scale mesocosm experiments with different number of JFSs.

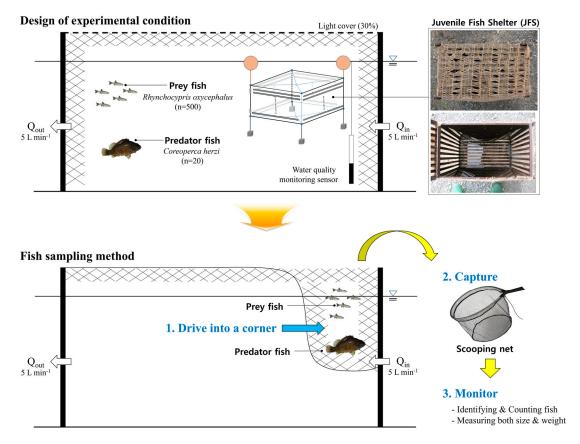


Figure 2. Experimental design of a juvenile fish shelter (JFS), and fish sampling method utilized in this study.

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Description		Control	T1	T2	Т3
	Water volume (m ³)	8.0	8.0	8.0	8.0
Test bed Physical condition	Water depth (cm)	80	80	80	80
	Input & output flow rate (L⋅min ⁻¹)	5.0	5.0	5.0	5.0
	Net size (mm)	2	2	2	2
	Light transmissivity (%)	30	30	30	30
	Number of patches (JFS)	0	1	2	4
Fish shelter	Patch volume (m ³)	0	0.08	0.15	0.30

Patch area (m^2)

Number of prey fish (individual) b

Size of prey fish (mm) b

Number of predator fish (individual) ^c

Size of predator fish (mm) ^c

0

500

 28.9 ± 1.0

20

 88.9 ± 2.0

0.25

500

 29.3 ± 0.9

20

 88.8 ± 1.5

0.50

500

 29.2 ± 0.9

20

 88.6 ± 1.3

1.00

500

 29.3 ± 0.9

20

 88.8 ± 2.1

Table 1. Experimental design and operating conditions of large-scale mesocosm experiments between August 2011 and May 2012 ^a.

Notes: ^a Experiment was conducted three times; Period 1: 5 August to 16 September 2011; Period 2: 30 September to 4 November 2011; Period 3: 6 April to 11 May 2012; ^b R. oxycephalus; ^c C. herzi.

2.2. Water Quality Analysis

Experimental fish

Raw water from the Nakdong river mainstream was utilized as the supply water with a continuous flow of 5 L·min $^{-1}$ in each experimental water channel. As displayed in Figure 2, the depth, electric conductivity (EC), dissolved oxygen (DO), pH, and temperature of water were measured hourly on site with portable water quality instruments (i.e., YSI 550A and YSI 63). Samples for the water quality analysis were collected in 2-L polyethylene bottles, transported to the laboratory, and analyzed using standard methods [27]. SS (gravimetric method), BOD (BOD electrode method), TN (UV absorption spectrophotometry), NH $_4$ ⁺ (indophenol method), NO $_3$ ⁻ (cadmium reduction method), TP (ascorbic reduction method), and PO $_4$ ³⁻ (ascorbic reduction method) were analyzed in the laboratory based on the standard methods [27].

Because both phytoplankton and zooplankton served as the prey's food source, concentration changes in both phytoplankton and zooplankton were also investigated during the experiment. Both phytoplankton and zooplankton were collected from each experimental site using a plankton net (mesh size: $64 \mu m$). The specimens were fixed in a 5% formalin solution, and microscopically examined on an S–R grid slide glass using an optical microscope (Zeiss Axioplan, Jena, Germany).

2.3. Experimental Fish

The native fish raised from an aquaculture farm in the Nakdong river basin were used as the experimental fish to simulate the natural fish communities of the Nakdong river and to control both size and density of prey and predator fish. Though JFS can be utilized for a variety of juvenile fish (or small size fish), the function of JFS for the conservation of local prey fish that inhabit in the Nakdong river was primarily evaluated in this study.

Based on a clear predator–prey relationship in local fish communities, two fish species were used in this experiment: *Coreoperca herzi* (*C. herzi*) as the predator and *Rhynchocypris oxycephalus* (*R. oxycephalus*) as the prey. *R. oxycephalus* belongs to the subfamily Cyprininae, which is mainly distributed in Republic of Korea, China, and Japan [28]. *R. oxycephalus* individuals generally become approximately 100 mm long at three years of maturity, and have long and cylindrical bodies. The staple diet of *R. oxycephalus* consists of algae, zooplankton, and mosquito larvae [29]. *R. oxycephalus* is widely dispersed in the Republic of Korea, inhabiting mountainous streams and rivers with various flow rates (i.e., 0.1–1.2 m·s⁻¹). *C. herzi* is a native piscivorous fish of the family Centropomidae. A three-year-old mature fish becomes approximately 100 mm long, and the adults can grow up to 250 mm long [30]. The main habitats for *C. herzi* are the middle and upstream regions with boulders and cobbles. *C. herzi* is an agile biting fish that is known for its aggression and its propensity to defend its territory within a radius of several meters [31].

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To evaluate the feasibility of the JFS as an effective shelter and habitat for juvenile fish, 500 individuals of *R. oxycephalus* were released into each experimental site along with 20 individuals of *C. herzi* predators (see Figure 2). Both initial size and weight of prey (i.e., *R. oxycephalus*) and predator (i.e., *C. herzi*) are summarized in Table 1.

The fish were monitored using a catch per unit effort (CPUE) method in all large-scale mesocosm experiments every week during the experimental period. As shown in Figure 2, fish were collected in the corner by adjusting the nylon net of each mesocosm and using scoop net after shifting of the JFS. After the identification and counting the fish, both body size and weight of *R. oxycephalus* and *C. herzi* were measured in separate tank with supplied oxygen. Finally, fish were returned to each mesocosm with different time intervals. No death or damages of collected fish was observed during these fishing gear and monitoring performance.

2.4. Quantification of the Predation Sequence

The predation sequence described by Fuiman and Magurran [32] is divided into three stages of encounter, attack, and capture, with each stage consisting of subsections with different characteristics. Vulnerability is defined as the probability of prey being consumed by certain predator, and the final vulnerability is determined based on the whole predation sequence [33]. Thus, the relationship between predator and prey can be described by Equation (1).

$$V = P_E \cdot P_A \cdot P_C \tag{1}$$

where V (vulnerability) is the probability of prey being consumed by predator, P_E (encounter rate) is the probability of encounter between predator and prey, P_A (attack rate) is the probability of attack by the predator, and P_C (capture success) is the probability of capture [32].

Since both vulnerability and prey survival are important variables to evaluate the performance of certain habitat (shelter) from predator [32,33], both vulnerability and prey survival for newely-developed JFSs were determined in a large-scale mesocosm experiment. Similar to other studies [32,33], the vulnerability of *R. oxycephalus* was directly estimated by monitoring consecutive feeding value of *R. oxycephalus* after being consumed by *C. herzi* every week. On the other hand, the prey survival of *R. oxycephalus* was estimated by monitoring the remaining number of *R. oxycephalus*.

Although both vulnerability and prey survival are based on the population changes in R. oxycephalus, vulnerability indicates the population changes directly consumed by predator, whereas prey survival indicates the population changes from all cases of death (e.g., disease, starvation etc.) including the consumption by predator. In this study, the attack probability of C. herzi against R. oxycephalus was assumed to be 100% [P_A (attack) = 1] because satiation conditions for predator fish rarely occur in a large-scale mesocosm experiment with no additional food sources [32,34].

2.5. Statistical Analysis

To establish a statistical evaluation of both prey survival and vulnerability with respect to both seasonal and shelter effects, a randomized complete block design (RCBD) was applied to prevent large differences in experimental units from making differences between treatment factor levels [35,36]. Since RCRD assumes that a population of experimental units can be divided into a number of relatively homogeneous blocks (i.e., elapsed time in this study), blocks generally represent sources of variation that are not related to the treatments (i.e., different experimental season and different numbers of JFS). Considering that experiment was replicated in a variety of conditions in different seasons, the variability needs to be removed so the actual effects of treatment (i.e., both seasonal and shelter effects) can be detected. Thus, the treatment effects can be split into three terms, and models for RCBD can be drawn as follows:

$$y_{iik} = \mu + \alpha_i + \beta_i + (\alpha \beta)_{ii} + b_k + \varepsilon_{ijk}, i = 1 - 4, j = 1 - 3, k = 1 - 5$$
 (2)

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where y_{ijk} is the response of the treatment, μ is the overall mean, α_i is the shelter effect, β_i is the seasonal effect, $(\alpha\beta)_{ij}$ is the interaction effect of shelter and season, b_k is the block effect, and ε_{ijk} is independent error.

In this study, multiple-way ANOVA with RCBD was performed with the Duncan option in PROC GLM (SAS® 9. 4, SAS Institute Inc., Cary, NC, USA) to determine the main effects of contributions from independent variables (i.e., different experimental season and different numbers of JFS), and to identify significant interactions among the independent variables. From the statistical analysis, both prey survival and vulnerability in relation to each experimental condition with different season and patch volume of JFSs were compared to determine the main effect of each independent variable.

3. Results and Discussion

3.1. Changes in the Water Quality

Water quality measurements were performed three times for each experimental period. As summarized in Table 2, there were no significant seasonal variations in most water quality parameters through the whole study, and relatively constant values except for the water temperature were observed. The changes in average water temperature were mainly attributed to the seasonal effect, and no, if any, significant seasonal variances in pH, DO, EC, SS, and BOD were noted for each experimental period.

Since the growth of phytoplankton has been reported to be significantly dependent upon changes in both concentration and chemical form of the nutrients [37,38], both the concentration and chemical form of nutrients were also observed. No significant seasonal variances in TN and TP were observed. However, the average phytoplankton densities significantly changed, and were measured to be 908 cells $\rm mL^{-1}$, 526 cells $\rm mL^{-1}$, and 712 cells $\rm mL^{-1}$ during Periods 1, 2, and 3, respectively. During the whole study, Bacillariophyceae was found to be dominant, accounting for 76.2% of total phytoplankton cells, and to be served as an abundant food for *R. oxycephalus*.

Similarly, the average zooplankton densities significantly changed, and were measured to be 5.3 ind L^{-1} , 2.8 ind L^{-1} , and 1.5 ind L^{-1} during Periods 1, 2, and 3, respectively. The average zooplankton densities became greater as the water temperature increased. During the whole study, rotifer density was found to be dominant, with a few cladocerans and copepods, and can be served as a major food for *R. oxycephalus*. In this study, both phyto- and zoo-plankton served as the only natural food for *R. oxycephalus*, and no other plankton feed was provided.

Table 2. Water quality changes in the large-scale mesocosm during the experimental periods.

Description	Period 1 ^c	Period 2 ^d	Period 3 ^e
Rainfall (mm)	108	460	98
Water depth (cm) ^a	84 ± 4	85 ± 5	84 ± 3
Water temperature (°C) ^a	25.6 ± 2.0	16.0 ± 2.5	19.7 ± 3.3
рН ^а	8.7 ± 0.6	9.2 ± 0.3	9.1 ± 0.3
DO (mg $O_2 L^{-1}$) ^a	11.4 ± 1.3	13.5 ± 1.7	12.7 ± 0.5
EC (μ S·cm ⁻¹) ^a	155 ± 17	187 ± 10	163 ± 11
SS (mg·L $^{-1}$) b	1.0 ± 0.2	0.9 ± 0.1	1.7 ± 0.6
BOD (mg $O_2 L^{-1}$) b	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
TN (mg N L ⁻¹) b	1.590 ± 0.090	1.510 ± 0.080	1.520 ± 0.050
NH_4^+ (mg $N L^{-1}$) b	0.114 ± 0.012	0.119 ± 0.009	0.131 ± 0.010
$NO_3^- (mg N L^{-1})^b$	1.390 ± 0.021	1.403 ± 0.040	1.419 ± 0.008
TP (mg P L ⁻¹) b	0.050 ± 0.004	0.049 ± 0.005	0.056 ± 0.001
PO_4^{3-} (mg P L ⁻¹) b	0.026 ± 0.001	0.023 ± 0.001	0.024 ± 0.002
Phytoplankton (cells mL^{-1}) b	908 ± 184	526 ± 159	712 ± 63
Zooplankton (ind L^{-1}) b	5.3 ± 2.8	2.8 ± 2.2	1.5 ± 0.6

Notes: a One hour interval data of water quality sensor (XLM6000, YSI) (n = 1090); b Laboratory analysis data determined by standard methods for Period 1 to 3; c Period 1: 5 August, 26 August, and 16 September 2011 (n = 3); d Period 2: 30 September, 21 October, and November 4, 2011 (n = 3); e Period 3: 6 April, 27 April, and 11 May 2012 (n = 3).

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3.2. Changes in Body Size and Weight

Over the entire study, the average body size and weight of *R. oxycephalus* ranged from 27.4 to 32.1 mm, and from 0.36 to 0.45 g, respectively, whereas the average body size and weight *of C. herzi* ranged from 84.5 to 95.3 mm, and from 14.9 to 23.5 g (see Table 3). These observations displayed that the body size of *R. oxycephalus* increased by 2.0%–8.0%, and its weight increased by 6.8%–12.2%. In contrast, the body size of *C. herzi* increased by 1.2%–3.1%, and its weight increased by 2.7%–12.4%.

In the case of *C. herzi*, the growth rate (i.e., 12.2%) of the body weight was greater in the control groups during Period 1. Due to the higher water temperature during Period 1, the predatory activity was expected to be greater. Since JFSs for juvenile fish were not installed in the control groups, both enhanced predation activity and absence of JFS may have facilitated the growth of *C. herzi*.

Since the survival of the prey was found to correlate significantly with the mouth gape size of the predators in predator–prey relationships, the mouth gape size of the predators is known to be an important factor in determining prey vulnerability [39]. From the previous study regarding the vulnerability of juvenile fish (*Clupea pallasii*, size 8–32 mm), the vulnerability was highest at a size of 20 mm, and decreased as the juvenile fish grew in size [32]. This correlation stems from the fact that the predator's gape size played an important role in determining prey vulnerability. Thus, prey fish have been reported to grow rapidly as a defense mechanism [40,41]. However, the rapid growth of juvenile fish as a defense mechanism was not clearly observed in this study.

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Table 3. Changes in body size and weight of both prey (*R. oxycephalus*) and predator (*C. herzi*) fish during each experimental period.

R. oxycephalus					C. herzi								
Description		IBS ^a (mm)	FBS ^b (mm)	GR ^c (%)	IBW ^d (g)	FBW ^e (g)	GR ^c (%)	IBS ^a (mm)	FBS ^b (mm)	GR ^c (%)	IBW ^d (g)	FBW ^e (g)	GR ^c (%)
	Control	28.5 ± 1.0	29.3 ± 0.7	2.6	0.39 ± 0.01	0.44 ± 0.01	10.4	88.7 ± 2.0	90.8 ± 2.8	2.4	18.5 ± 0.5	21.2 ± 1.7	12.4
	T1	28.7 ± 0.9	29.9 ± 0.7	3.9	0.39 ± 0.01	0.44 ± 0.01	10.0	89.2 ± 1.7	91.5 ± 2.2	2.6	18.7 ± 0.4	20.8 ± 1.2	9.8
Period 1	T2	28.6 ± 1.0	30.1 ± 0.6	5.1	0.39 ± 0.01	0.44 ± 0.01	10.1	89.0 ± 1.6	90.8 ± 2.0	2.0	18.3 ± 0.4	20.6 ± 1.3	11.0
	Т3	28.6 ± 0.9	30.2 ± 0.7	5.1	0.39 ± 0.01	0.45 ± 0.01	12.2	88.9 ± 2.3	90.7 ± 2.2	2.0	18.5 ± 0.6	20.3 ± 1.5	8.9
Period 2	Control	30.7 ± 1.0	31.3 ± 0.9	2.0	0.40 ± 0.01	0.45 ± 0.01	10.8	93.4 ± 1.8	94.5 ± 2.3	1.2	22.1 ± 0.9	23.5 ± 1.5	6.1
	T1	30.5 ± 0.9	31.5 ± 0.9	3.2	0.40 ± 0.01	0.45 ± 0.01	11.2	92.7 ± 1.4	94.9 ± 1.7	2.2	22.2 ± 0.8	23.4 ± 0.9	5.0
	T2	30.5 ± 0.7	32.1 ± 0.8	5.0	0.40 ± 0.01	0.45 ± 0.01	10.7	92.1 ± 1.6	94.8 ± 2.1	2.9	22.0 ± 1.0	23.3 ± 1.3	5.7
	Т3	30.7 ± 0.9	31.7 ± 0.7	3.4	0.40 ± 0.01	0.45 ± 0.01	10.5	92.3 ± 2.0	95.3 ± 2.1	3.1	22.2 ± 0.6	23.4 ± 1.0	5.0
Period 3	Control	27.4 ± 1.0	28.0 ± 0.8	2.2	0.36 ± 0.01	0.39 ± 0.01	7.6	84.7 ± 3.0	87.4 ± 2.2	3.1	15.0 ± 0.5	15.9 ± 0.6	5.5
	T1	28.7 ± 0.9	29.9 ± 0.7	3.9	0.37 ± 0.01	0.40 ± 0.01	6.8	84.5 ± 1.5	86.9 ± 1.2	2.8	14.9 ± 0.4	15.8 ± 0.6	5.2
	T2	28.6 ± 1.0	30.1 ± 0.6	5.1	0.37 ± 0.01	0.41 ± 0.01	8.1	84.8 ± 0.7	87.2 ± 1.3	2.7	15.1 ± 0.4	15.7 ± 0.5	3.6
	T3	28.6 ± 0.9	31.1 ± 0.8	8.0	0.37 ± 0.01	0.41 ± 0.01	8.6	85.1 ± 2.0	87.2 ± 1.8	2.4	15.2 ± 0.6	15.6 ± 0.6	2.7

Notes: ^a IBS: Initial body size; ^b FBS: Final body size; ^c GR: Growth rate; ^d IBW: Initial body weight; ^e FBW: Final body weight.

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3.3. Prey Survival Results

During the experiment, the population of prey fish (*R. oxycephalus*) decreased with time, as shown in Figure 3. In the control group with no JFS, the population of prey fish decreased significantly. Since six weeks is not enough time for prey fish to die naturally, the decrease in the population of prey fish was mainly attributed to the predation by predator fish (*C. herzi*). The experimental results obtained during three periods of this study indicated that the prey survival of *R. oxycephalus* consistently increased as the patch volume of JFS increased, regardless of the experimental period (see Figure 4). In the control group with no JFS, the average prey survival ranged from 19.6% to 36.9%. Using a patch volume of 0.08 m³ (T1), the average prey survival ranged from 35.2% to 54.6%. The average prey survival was 47.2%–64.2% for a patch volume of 0.15 m³ (T2) and 66.3%–72.8% for a patch volume of 0.30 m³ (T3), respectively, indicating a positive correlation between prey survival and patch volume was obtained.

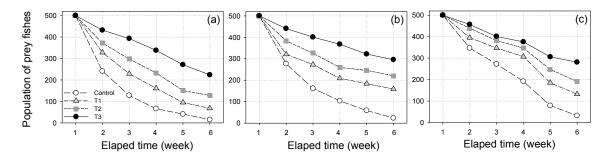


Figure 3. Variation in population of prey fish over six weeks: (a) Period 1; (b) Period 2; and (c) Period 3.

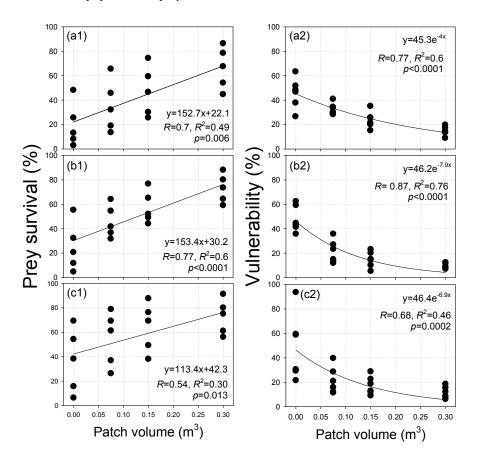


Figure 4. (1) Changes in prey survival with various patch volumes of JFS; and (2) changes in vulnerability with various patch volumes of JFS: (a) Period 1; (b) Period 2; and (c) Period 3.

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The seasonal maximum prey survival was $66.3\% \pm 17.1\%$ for the summer (Period 1), $73.1\% \pm 11.7\%$ for the autumn (Period 2), and $72.8\% \pm 14.3\%$ for the spring (Period 3). Prey survival displayed seasonal differences due to both different aquatic environmental factors (i.e., changes in water quality and biological communities) and different behavior of the experimental fish with various water temperatures. The water temperature significantly varied by season, with values of 25.6 ± 2.0 °C in summer, 16.0 ± 2.5 °C in autumn, and 19.7 ± 3.3 °C in spring. These differences may have directly affected the fish behavior, since fish are very sensitive to water temperature [42], and low temperatures inhibit their growth rates and feeding activities [43,44]. Considering *C. herzi* is active during the summer [31] and *R. oxycephalus* prefers to relatively colder water temperatures, the feeding of *C. herzi* was more active and the prey survival was lower during the summer. In contrast, during autumn, when the water temperatures were relatively lower, the feeding activity of *C. herzi* decreased whereas that of *R. oxycephalus* increased, leading to increased predator avoidance and enhanced prey survival.

3.4. Statistical Analysis Results

As summarized in Table 4, two main effects were statistically significant (p < 0.05) for both prey survival and vulnerability, indicating that both prey survival and vulnerability were significantly affected by both seasonal and shelter variations. Statistically, the JFS developed in this study was revealed to improve the survival rate of juvenile fish, and to decrease the vulnerability of juvenile fish in a freshwater ecosystem. In addition, both prey survival and vulnerability displayed seasonal differences due to both different aquatic environmental factors (i.e., changes in water quality and biological communities) and different behavior of the experimental fish with various water temperatures. However, the interaction between two treatments (i.e., shelter and season) for both prey survival and vulnerability was negligible (p = 0.089) at significance level of 0.05, indicating that the combined effect of seasonal and shelter variations on both prey survival and vulnerability was not statistically significant.

Table 4. ANOVA table for randomized complete block design (RCBD) in terms of prey survival and vulnerability.

		Prey S	Survival		Vulnerability				
Sources of Variation	df	MS	F	Pr > F	df	MS	F	Pr > F	
Seasonal effects	2	5069.8	181.04	< 0.0001	2	3050.9	42.95	< 0.0001	
Shelter effects	3	1134.7	40.52	< 0.0001	3	390.7	5.50	0.0074	
Interaction	6	55.4	1.98	0.089	6	29.5	0.42	0.8645	
Elapsed time	4	3836.6	137.01	< 0.0001	4	185.2	2.61	0.0484	
Error	44	28.0			44	71.0			
Total	59				59				

Notes: Acronyms used: df = degree of freedom, MS = mean square, F = F-statistic value, Pr = p-value.

3.5. Performance Evaluation of the Developed JFS in Terms of Vulnerability

Predation decreases prey density, and impacts the survival rate of the prey due to interspecies competition and avoidance behaviors among prey [45]. Thus, prey fish require an effective method (e.g., shift in habitats and change in activity time) to decrease the probability of encounter with piscivorous predator fish [46–48]. Considering capture process is a complex function of the sensory and motor systems of fish [33,49,50], $P_{\rm C}$ in Equation (1) could not be readily estimated.

In the case of Eurasian perch (*Perca fluviatilis*), which is closely related to *C. herzi*, 60%–100% capture probability was estimated for conditions similar to this study (i.e., predator size, 66–157 mm; prey size, 30 mm) with a stalking approach within a proximity of 2–5 cm [51]. Thus, the capture probability of *C. herzi* was assumed to be around 80% [P_C (capture success) = 0.8] in this study. Finally, the probability of encounter (P_E) was calculated based on Equation (1) after the vulnerability (V) of *R. oxycephalus* was directly estimated by monitoring the consumed number of *R. oxycephalus* by *C. herzi*.

As displayed in Figure 4, the vulnerability was highest in the control conditions with no JFS during Periods 1 to 3, resulting in a greater predation danger for the prey. As is also evident in

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Figure 4, the vulnerability presented an exponential decay with high values of correlation coefficient (R^2 = 0.46–0.76) as the patch volume of JFS increased. In addition, seasonal effects were found in the JFS with maximum patch volume condition, where a higher predation activity was observed during the summer (14.8% \pm 3.7%) compared with the predation activity during the autumn (9.5% \pm 2.2%) or spring (11.6% \pm 4.8%).

As also displayed in Figure 4, the correlation between prey survival and patch volume yielded significant relationships with $R^2 = 0.30$ –0.60 (p < 0.05). By contrast, the level of vulnerability exponentially decreased as the JFS volume increased (p < 0.05). The average values of vulnerability ranged from 40.0% to 49.7% under control conditions with no JFS, but they decreased from 9.9% to 14.8% with JFS volumes of 0.3 m³. Based on these results, the JFS developed in this study was found to improve the survival rates of juvenile fish in a freshwater ecosystem, although many other environmental factors may also affect the survival rates of juvenile fish. Similar to this study, other studies have also shown that the risk of being preyed upon was found to be low with artificial shelters [22], and the survival rates of juvenile fish improved with the increase in the structural complexity of the habitats [22,52–54].

Considering that an increased patch volume of JFS creates more shelter for juvenile fish under the significant predatory pressure, juvenile fish switched habitats to JFS, leading to increased survival and lower vulnerability. From these results, the increase in patch volume of JFS had a direct effect on the survival of the juvenile fish, which is presumably attributed to the decrease in the number of predator encounters [55]. Although the vulnerability is a complex function of encounter, attack, and capture processes, the increase in patch volume of JFS definitely provide hiding spots for juvenile fish; hence, the possibility of direct encounter between predator and prey can be reduced when the JFS is present.

3.6. Correlation of Vulnerability and Prey Survival

As displayed in Figure 5, the relationship between vulnerability and prey survival exhibited an exponential decay with $R^2 = 0.56$ (p < 0.0001). Aforementioned above, the change in vulnerability was mainly attributed to the decreased number of encounters; therefore, the presence of JFS directly affected the prey survival. Under natural conditions, prey fish take refuge in structurally complex environments, such as dense plants or reefs within the littoral region, to reduce their encounters with predators [52–54]. Therefore, juvenile fish need to sufficiently utilize the structural complexity of their habitats to avoid predators [56]. However, predators also adapt certain strategies to their current living conditions such as the ambushing behaviors of *Micropterus salmoides* or *Esox lucius* [25,57]. Considering that most prey (i.e., juvenile fish) simply engage in shoaling or form schools [58] in open waters without shelters to suppress encounters with predators, JFSs developed in this study can be used to attract more juvenile fish, to suppress encounters with predators, and finally to increase the survival rates of juvenile fish. Therefore, the deployment of JFSs can be a practical alternative for effective fishery resources management to improve species diversity and to conserve fish communities in a freshwater ecosystem.

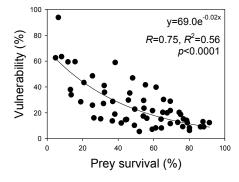


Figure 5. Correlation of vulnerability and prey survival drawn in this study.

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4. Conclusions

To investigate the feasibility of the developed juvenile fish shelter (JFS) to improve the survival rates of juvenile fish under predation by Korean native piscivorous fish, the evaluation of the developed JFS using a large-scale mesocosm experiment was performed for two years. Through the whole study, there was no significant seasonal variations in most water quality parameters, and relatively constant values except for the water temperature were observed. From the experimental results, the prey survival of *R. oxycephalus* consistently increased as the patch volume of JFS increased, regardless of the experimental period. Statistically, the JFS developed in this study was found to improve the survival rate of juvenile fish in a freshwater ecosystem, although many other environmental factors may also affect the survival rate of juvenile fish. In addition, both prey survival and vulnerability were statistically related to both seasonal and shelter variations. Thus, JFSs developed in this study can be used to attract more juvenile fish, to suppress encounters with predators, and finally to increase the survival rate of juvenile fish. Although further study are required for natural rivers and lakes with different physicochemical conditions, the deployment of JFSs can be a practical alternative for effective fishery resources management to improve species diversity and to conserve fish communities in a freshwater ecosystem.

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