低温がコイに及ぼす麻酔効果

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Short- and Long-term Cold-anesthesia in Carp

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The efficacy of cold anesthesia in the transportation of live fish was evaluated using carp acclimated at 23°C. The carp could be safely maintained in an anesthetic state for 5 h in water kept at 4°C and in the anesthetized or sedate state for 24 h at 8–14°C. Some anesthetized carp showed signs of convulsion when they received external stimuli, and bled mainly from gills. Hemorrhage became distinct with the decrease in temperature and the duration of the cold treatment. The sedate carp showed no such excitement and bleeding. The sedate state is considered to be adequate for transportation, regardless of the anesthetic time; 14°C seems to be the optimal temperature when the carp are acclimated to 23°C.

In previous reports,[1,2] we studied the use of CO_2 as an anesthetic for the transportation of live fish, and reported that CO_2 has two drawbacks in use as an anesthetic; the induction time is relatively long and its therapeutic range is relatively narrow. To overcome these drawbacks, we used two concentrations of CO_2 in various combinations: a high concentration for the rapid induction of anesthesia and a low concentration for the maintenance of anesthesia.[2] As a result, the induction time could be successfully shortened by raising the concentration of CO_2 used as the high concentration; but, not all the carp could be maintained in a favorable anesthetic state for 10 h although they could be anesthetized or sedated by using high ($P_{CO_2} \fallingdotseq 250$ mmHg) and low ($P_{CO_2} \fallingdotseq 100$ mmHg) partial pressures of CO_2. In these studies, we used adult carp *Cyprinus carpio* that were acclimated to a water temperature of 23±1°C, and anesthetized in water containing high concentrations of CO_2. If the water temperature decreases, the concentration of CO_2 needed for the induction and maintenance of anesthesia will also probably decrease and its effective range may be widened. Moreover, low temperatures are also known to have an anesthetic action on fish.[3–5]

Many anesthetics have been used in the transportation of live fish,[6–8] but most of them are not safe. On the other hand, a low temperature is completely harmless regarding humans or fish. Cold anesthesia as well as CO_2 anesthesia seems to be useful in transporting fish for human consumption. However, cold anesthesia has been used only as a short-term, convenient method.[9–13] Therefore, in this study prior to the experiment on the anesthetic method combining CO_2 with low temperatures, we evaluated the anesthetic effects of low temperatures on fish.

Materials and Methods

Adult carp *Cyprinus carpio*, each weighing 492±36 g (mean±SD, $n=460$), were used. Each group of ten carp was reared in a 1,000 *l* plastic tank placed indoors, and acclimated to the condition of 23±1°C under a 14 h-light and 10 h-dark cycle for at least one month. They were daily fed on a commercial diet, but were given no food the two days prior to the beginning of the experiment.

Initially, each group of ten carp was lightly anesthetized with MS222 (tricaine methanesulfonate), and then transferred into an experimental cage, composed of ten compartments of five rows and two tires and down and made of an acrylic fiber plate containing holes ($\phi \fallingdotseq 1$ cm) for water circulation. One carp was placed in one compartment (9.5×35×9.5 cm), and the experimental cage containing the ten carp was immersed in water of a container (65×36×30 cm, 30 *l*) kept at 23±1°C; ten carp were placed in the container for simulation of transportation. In this study we used two containers, that is, for cold treatment at low temperatures and for acclimation or recovery. The water in these containers was extensively aerated and filtrated.

In the case of short-term anesthesia, the carp were acclimated in a treatment container for at

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Table 1. Anesthetic stage for cold anesthesia in carp

Anesthetic stage	Opercular movement	Swimming motion	The sense of equilibrium
0	Normal	Normal	Normal
LS*1	Weak	Normal	Normal
DS*2	Weak	Normal	Normal
I	Weak	Partial loss	Partial loss
II	Weak	Partial loss	Total loss
III	Weak	Total loss	Total loss
IV	Very weak	Total loss	Total loss
V	Stop	Total	Total

*1 LS: Light sedation.
*2 DS: Deep sedation. See the text as to the difference between LS and DS.

least 10 h with the exception of a direct-transfer experiment. The water temperature of the hypothermic container was decreased at three rates (Table 2) from 23°C to 2°C; in a preliminary experiment, we confirmed in advance that all the carp became anesthetized at about 3°C. In the direct-transfer experiment, the carp were acclimated in an acclimation container kept at 23±1°C for approximately one hour, and the experimental cage containing ten carp was then directly transferred into the hypothermic container at 2°C for 30 min. Immediately after the water temperature of the hypothermic container reached 2°C, or after a 30-min exposure to 2°C, the ambient temperature was returned, to determine the time and temperature required to revive, that is, from 2°C to 23°C at five rates (Table 2).

In a case of long-term anesthesia, the carp were acclimated in the acclimation container kept at 23±1°C for approximately one hour, and the experimental cage containing ten carp was then transferred into the hypothermic container. In some experiments, the water temperature was changed during the cold exposure. The anesthetic time was 5, 10, or 24 h. After being exposed to low temperatures, the carp were returned to the recovery container kept at 23±1°C.

To control the water temperature of the containers, two thermoregulators (TAIYO-KAGAKU-KOGYO, CL-30), six flexible coolers (TOKYO-RIKA, EC-10), and six heaters (500 W) for tropical fish were used in several combinations. During the cold treatment, changes in the degree of anesthetic state in the carp were measured in anesthetic stages (Table 1), which were defined for cold anesthesia. Following exposure to low temperatures, the recovery rate in each experiment was examined. When a 100% recovery rate was obtained, we returned the carp to the rearing tank for one week.

Results

Results of Short-term Cold Anesthesia

When the ambient water temperature was rapidly lowered, the carp passed through a sequence of anesthetic stages from Stages 0 to V (Table 1) and showed a characteristic behavior. Concomitantly with a decrease in the water temperature, the respiration, opercular movement, and swimming motion in the carp were gradually depressed (Stage LS, Light sedation). They became unresponsive to external visual and tactile stimuli (Stage DS, Deep sedation), but the induction of this sedate state was not clear. Over time, they partially lost their sense of equilibrium and repeatedly lost and regained consciousness (Stage I). At the same time, they began to show excited and violent behavior. This excitement continued throughout the period from Stage I to II. Next, they inverted themselves completely (Stage II). They rapidly progressed from Stage II to V; after achieving Stage II, they soon ceased all motion except for a weak opercular movement (Stage III), showed only slight opercular movement (Stage IV), and finally stopped breathing (Stage V). However, some carp even at Stage V sometimes showed violent movement when they received external stimuli. On rewarming the water temperature, the fish regained consciousness.

To determine an adequate condition for short-term cold anesthesia, the relationship between the induction time of anesthesia and rate of decrease in water temperature, and the relationship between the recovery time from anesthesia and rate of increase in water temperature were examined. In a preliminary experiment, all the carp became anesthetized at Stage II or higher at 2°C or above. The induction time of anesthesia could be shortened with a decreased rate in water temperature, that is, 49±8 min at 0.30–0.32°C/min, 32±5 min at 0.52–0.54°C/min, and 7±3 min at the direct-transfer experiment, respectively (Table 2). The temperatures needed for inducing the carp into Stage II were 5.4±1.7°C at 0.30–0.32°C/min and 4.4±1.9°C at 0.52–0.54°C/min. This difference was significant ($p<0.005$).

With an increased rate in water temperature, the recovery time from anesthesia was also shortened (Table 2). When the carp were directly transferred from 2°C to 23°C, the recovery time from

Table 2. Results of short-term cold anesthesia in carp

Exp.*1 No.	*n*	Decreasing rate of temperature (°C/min)	Temperature needed for Stage II (°C) mean±SD	Time needed for Stage II (min) mean±SD	Increasing rate of temperature (°C/min)	Recovery temperature (°C) mean±SD	Recovery time (min) mean±SD	Recovery rate (%)
1–I	10				0.04	12.6±0.7	191±15	100
II	10				0.15	15.2±1.6	81±12	100
III	10	0.30–0.32	5.4±1.7	49±8	0.32	17.1±2.3	47±8	100
IV	10				0.67	20.0±1.2	27±5	100
V	10				Direct transfer*2	—	18±6	100
2–I	10				0.03	11.1±1.5	147±32	100
II	10				0.15	13.8±1.1	73±8	100
III	10	0.52–0.54	4.4±1.9	32±5	0.28	16.7±2.6	53±12	100
IV	10				0.69	18.8±1.3	24±2	100
V	10				Direct transfer*2	—	10±3	100
3–I	10				0.05	15.2±2.7	208±71	100
II	10				0.16	16.2±1.9	77±12	100
III	10	Direct transfer*2	—	7±3	0.29	18.5±1.6	56±6	100
IV	10				0.70	23	49±13	100
V	10				Direct transfer*2	—	18±5	100

*1 In Exps. 1 and 2, the temperature of the treatment container, in which ten carp were kept, was decreased from 23°C to 2°C and then soon increased from 2°C to 23°C. On the other hand, in Exp. 3, ten carp in the acclimation container kept at 23°C were transferred into the treatment container kept at 2°C and anesthetized for 30 min.

*2 Direct transfer denotes that the experimental cage containing ten carp was directly transferred from 23°C to 2°C or from 2°C to 23°C.

Table 3. Results of 24 h cold anesthesia in carp

Exp. No.	*n*	Temperature* for anesthesia (°C)	Time needed for Stage II (min) mean±SD	Percentage of carp in Stage II or higher at (h)					Recovery rate (%)
				0.5	3	6	12	24	
4	20	23	—	—	—	—	—	—	100
5–I	10	2		100	100	100	100	100	0
II	10	2→6	7±4	100	100	100	100	100	60
III	10	2→8		100	70	60	0	30	60
6–I	10	4		100	100	100	100	100	0
II	10	4→6	9±3	100	100	100	100	100	80
III	10	4→8		100	90	40	10	10	100
7–I	10	6		100	100	100	90	80	70
II	10	6→8	—	70	60	60	20	20	100

* a→b: The carp were cold-anesthetized at a °C for the first 30 min and at b °C for the following 23.5 h.

anesthesia was the shortest. In every experiment, all the carp recovered from anesthesia. Judging from the shortest induction and recovery time, however, it was concluded that direct transfer from 23°C to 2°C and from 2°C to 23°C is the best condition for short-term anesthesia, the induction time being 7±3 min and the recovery time being 18±5 min.

Results of 24-h Cold Anesthesia

To ascertain how long the carp can be maintained in an anesthetic state by cold anesthesia, the carp were initially cold-anesthetized for 24 h. According to the results obtained in the short-term anesthesia (Exps. 1–3), the carp were directly transferred from 23°C to 2, 4 or 6°C and treated for 30 min. For the following 23.5 h, they were exposed to 2, 4, 6, or 8°C to maintain the anesthesia. When the carp were directly transferred from 23°C to a low temperature or when the water temperature was changed during exposure to cold, the water temperature was con-

Table 4. Changes of the anesthetic stage in carp anesthetized with low temperatures for 24 h

Exp. No.	Temperature* for anesthesia (°C)	Percentage of carp in each anesthetic stage at (h)																															
		0.5					3					6					12					24											
		0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V
5-I	2						100						100						100						100						100						100
II	2→6						100					90	10					70	30					40	30					40	30					30	30
III	2→8						100	10	20	10	20	40		40		20	40			80	20					60	10			20			90			10	
6-I	4						100						100						100						100						100						100
II	4→6				20	40	40				40	40	20				30	60	10				30	70					60	40							
III	4→8						100	10			10	40	40	60			10	10	20	90				10		90				10						
7-I	6				10	50	40					40	60				20	40	40				50	20	30				30	40	30						
II	6→8	30			50	20		20	20			40	20	40			20	20	20	80				20		80				20							

* a→b: The carp were cold-anesthetized or cold-treated at a °C for the first 30 min and at b °C for the following 23.5 h.

trolled by the addition of small amount of cracked ice or water kept at 23°C. Table 3 summarizes the experimental results. The induction time needed for Stage II of anesthesia was 7±4 min at 2°C and 9±3 min at 4°C, but at 6°C, 15% of the carp did not achieve Stage II within a 30-min treatment. A temperature of 4°C seems suitable for the induction of anesthesia because all the carp achieved the favorable anesthetic stage, Stage II, or higher in a short time and because it gives less thermal stress to fish than 2°C does. As shown in Table 3, we could not safely anesthetize the carp for 24 h; some carp died after the cold exposure and some carp regained consciousness during the treatment. All the carp that were confined in a small container kept at 23±1°C for 24 h, survived with no apparent ill effects, suggesting that the death of the carp was due to only thermal stress. Table 4 shows the changes in the anesthetic stage of the carp. As expected, the carp exposed to a lower temperature became more deeply anesthetized.

As mentioned above, when the ambient temperature was lowered, the carp first became sedate and then became anesthetized. The sedate state can be divided into two states: light sedation in which the carp hardly moved except for weak opercular movement and deep sedation in which they did not respond to mechanical stimulation. Therefore, we next examined whether or not the carp can be maintained in the sedate state for 24 h. They were exposed to 8–18°C because 30% of the carp died at 6°C whereas no carp died at 4 → 8°C and at 6 → 8°C (Exps. 6-III and 7-I, II in Table 3). Table 5 summarizes the results. Some carp became conscious at 16°C and at 18°C, but all the carp could be maintained in the anesthetized or sedate state for 24 h at 8–14°C; even if they were cold-anesthetized at 4°C for the first 30 min, they all survived (Exps. 9 and 10 in Table 5). The survival rate after a week was 100%. Table 6 shows the changes of the anesthetic stage. At 12–14°C, the carp were maintained in the sedate state with the exception of an initial period.

Results of 10-h Cold Anesthesia

Next, the time of cold anesthesia was shortened from 24 h to 10 h. We chose 2°C or 4°C as a temperature for the induction of anesthesia and 2, 4, or 6°C as a temperature for the maintenance of anesthesia. Table 7 summarizes the experimental results. Irrespective of the temperature for the

Table 6. Changes of the anesthetic stage in carp treated with low temperatures for 24 h

Exp. No.	Temperature* for cold treatments (°C)	Percentage of carp in each anesthetic stage or sedate state at (h) 0.5								3								6								12								24							
		0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V
8—I	8			70	10			20				70			10	10	10			70	30							80	20						50	50					
II	12		30	50		10	10				50	50							30	70							50	50							40	60					
III	14		40	50			10				50	50							20	80							30	70							20	80					
IV	16		50	50						30	10	60							40	60							10	90							10	90					
V	18	30	40	20	10					40	20	40						30	40	30						30	20	50						50	30	20					
10	4→8								100			10			20	20	50			40			20	40				40			40	20			20	40			30	10	

* a→b: The carp were cold-anesthetized at a °C for the first 30 min and at b °C for the following 23.5 h.

Table 8. Changes of the anesthetic stage in carp treated with low temperatures for 10 h or 5 h

Exp. No.	Temperature* for cold anesthesia (°C)	Percentage of carp in anesthetic stage or sedate state at (h) 0.5								1								3								5								10							
		0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V	0	LS	DS	I	II	III	IV	V
11—I	2							30	70							20	80							10	90								100								100
II	2→4							10	90							20	80							50	50							40	60							60	40
III	2→6							15	85						40	50	10			15			20	50	15			10	5		5	60	20		5	20	10	5		50	10
12—I	4						10	20	70							30	70							20	80							30	70							10	90
II	4→6						5	40	55					5	10	65	20					5	20	45	30			5	5	5	10	50	25			20		10	15	40	15
13—I	2						10	40	50							30	70							20	80								100								
II	4→4						6	60	34						6	54	40						4	54	42						4	50	46								

* a→b: The carp were cold-anesthetized at a °C for the first 30 min and at b °C for the following 9.5 h (Exps. 11 and 12) or 4.5 h (exp. 13).

Table 5. Survival rate of carp treated with low temperatures for 24 h

Exp. No.	*n*	Temperature* for cold treatment (°C)	Percentage of anesthetized or sedate carp at (h)					Survival rate (%) after 1 week
			0.5	3	6	12	24	
8–I	10	8	100	100	100	100	100	100
II	10	12	100	100	100	100	100	100
III	10	14	100	100	100	100	100	100
IV	10	16	100	70	100	100	100	100
V	10	18	70	60	70	70	50	100
9	20	4→23	—	—	—	—	—	100
10	10	4→8	100	100	100	100	100	100

* a→b: The carp were cold-anesthetized or cold-treated at a °C for the first 30 min and at b °C for the following 23.5 h.

Table 7. Results of 10 h or 5 h cold anesthesia in carp

Exp. No.	*n*	Temperature* for anesthesia (°C)	Time needed for Stage II (min) mean±SD	Percentage of carp in Stage II or higher at (h)					Recovery time (min) mean±SD	Recovery rate (%)	Survival rate after 1 week (%)
				0.5	1	3	5	10			
11–I	10	2		100	100	100	100	100	—	20	—
II	10	2→4	12±7	100	100	100	100	100	—	100	70
III	20	2→6		100	100	85	90	65	—	100	100
12–I	10	4	15±7	100	100	100	100	100	—	90	—
II	20	4→6		100	100	100	90	80	—	100	100
13–I	10	2	13±7	100	100	100	100	—	—	80	—
II	50	4→4	17±7	100	100	100	100	—	15±13	100	100

* a→b: The carp were cold-anesthetized at a °C for the first 30 min and at b °C for the following 9.5 h (Exps. 11 and 12) or 4.5 h (Exp. 13).

first cold exposure, some carp died during the experiment or within a week after they were exposed again to temperatures less than 6°C. All the carp recovered from anesthesia and survived for a week when they were later exposed to 6°C, but some carp recovered from anesthesia during the exposure. Even when the time of hypothermia was shortened to 10 h, all the carp could not be maintained in the favorable anesthetic state, Stage II or higher. However, the lowest temperature, at which they could be sedated, decreased to 6°C (Exp. 12–II in Tables 7 and 8) while in the case of 24-h cold anesthesia, some carp died at 6°C.

Results of 5-h Cold Anesthesia

It was possible to maintain the carp in the anesthetic stage during hypothermia as long when the anesthetic time was 5 h. By maintaining the temperature of the ambient water at 4°C, all fifty carp became anesthetized at Stage II or higher in five experiments (Exp. 13–II in Tables 7 and 8). Both the recovery rate after hypothermia and the survival rate after a week were 100%. However, the range of the effective temperatures necessary to safely anesthetize the carp for 5 h was considerably narrow; 20% of the carp did not recover from anesthesia at 2°C (Exp. 13–I in Tables 7 and 8). Moreover, the anesthetic stage was rather deep, being Stage III to V (Table 8); this probably suggests that the carp could survive 4°C only for 5 h.

Discussion

Cold anesthesia is a simple anesthetic method; the fish become easily anesthetized only when the ambient temperature is rapidly lowered to a certain degree or when the fish are put directly into water kept at a low temperature. In addition, a low temperature itself is safe for humans and fish. Despite these advantage, cold anesthesia has not been given much notice, probably from fear that a rapid change in the ambient temperature may produce deleterious thermal damage in fish. In fact, cold anesthesia has some serious drawbacks. For example, bleeding from gills, blood between scales, and face congestion were sometimes observed in carp during hypothermia. This hemorrhage tended to become marked with the decrease of temperature, duration of hypothermia, and progression of the anesthetic state

in the carp. Moreover, the carp in relatively superficial stages as well as in deep stages of cold anesthesia died, although, as previously reported,[1,2] most of the dead carp anesthetized with CO_2 were in Stage V at the end of anesthesia. This indicates that cold anesthesia affects the carp more severely than does CO_2 anesthesia with the same anesthetic effect.

Cold anesthesia also has other drawbacks. In this study, we used carp that were acclimated at 23 ± 1°C. They became anesthetized within 30 min when they were directly transferred from 23°C to 4°C or below. The range of the effective temperatures is extremely narrow and the temperature at which cold anesthesia is induced is extremely low. Chung[4] and Mittal and Whitear[13] suggested that the temperature at which cold anesthesia depends upon the acclimation temperature and fish species. If the carp are acclimated at temperatures much lower than 23°C, they will no longer become anesthetized, that is, the use of cold anesthesia is limited to fish acclimated at higher temperatures in the summer, as pointed out by Mittal and Whitear.[13] Cold anesthesia, before use, must be tested on other species which are sensitive to low temperatures or a marked change of the ambient temperature. McFarland and Klontz[9] reported that most fish can not tolerate a rapid change in water temperature of more than 5–10°C. In a preliminary experiment, porgies *Pagrus major* were cold-anesthetized at low temperatures in the same manner as the short-term anesthesia performed in the present study, but they were highly sensitive to low temperatures and most of them could not survive low temperature stress.*

It can be assumed that cold anesthesia is induced by a decrease in body temperature rather than by a water temperature of a certain degree. In this study, we demonstrated that a higher rate of decrease in water temperature brought about a significant decrease in the temperature at which cold anesthesia is induced. It is likely that the body temperature of carp at induction of anesthesia is almost the same at both of the two decreasing rates, and that a more rapidly decreasing rate can give rise to a larger difference between body temperature and water temperature. At recovery the same interpretation would be applicable. In a preliminary experiment using porgies, we found that the body temperature in each anesthetic stage was not significantly different irrespective of the rates of decrease in water temperature.* Prosser and Fahri[14] and Roots and Prosser[15] found that lowering the temperature of fish blocked reflexes and eventually nerve conduction. Its mechanism of action is probably due to a reduction of brain activity.

Initially, we defined Stage II or higher as the suitable anesthetic induction stage for the transportation of live fish. In the previous report,[1] we defined the anesthetic stages for CO_2 anesthesia using three criteria and considered that Stages III–IV were the suitable anesthetic stage for transportation. Compared with CO_2 anesthesia, each anesthetic stage of cold anesthesia is somewhat deeper due to the sedating action of low temperatures. For example, the carp in Stage I of CO_2 anesthesia showed only a partial loss of equilibrium, but the carp in Stage I of cold anesthesia showed a partial loss of swimming motion and weak opercular movement besides a partial loss of equilibrium. Consequently, Stage II of cold anesthesia is almost equivalent to Stage III of CO_2 anesthesia. Therefore, in this study we tried to maintain the carp in Stage II or higher, by cold anesthesia for 24 h, 10 h, or 5 h which corresponds to a long, middle, or short distance transportation, respectively. However, the carp could be safely maintained in the favorable anesthetic stage for 5 h but not for more than 10 h. The carp in the anesthetic state sometimes showed convulsive actions during hypothermia when they received mechanical stimulation. This suggests that the anesthetic action of low temperatures can not sufficiently restrain the excitement and mobility of the fish.

When the ambient temperature was rapidly lowered, the carp became sedate. In other words, the metabolic rate, heart rate, ventilation rate of the fish became restrained, as reported by Moffitt and Crawshaw[16] and Yamamitsu and Itazawa.[5] The carp in the lightly sedate state showed weak opercular movement and remained stationary at the bottom of the compartment, showing a slight body movement to keep their equilibrium. To distinguish the sedate carp from the conscious carp, we hit or vibrated the container. At this time, the lightly sedate carp showed only a slight avoidance motion without any excitement, whereas the carp in the deeply sedate state did not respond to the mechanical stimulation. An anesthetic is generally used for restraining the hyperactivity and oxygen consumption of the fish, but the

* unpublished data.

degree of restraint is important. The problems to be solved for the transportation of live fish are mainly regulation of the ambient water and undue injury of fish due to excitement. The activity and excitement of fish can be sufficiently prevented by the sedating effect of low temperatures. In fact, this sedating effect has been practically used in the transportation.[17] As for cold anesthesia, the anesthetized state is not appropriate, but the sedate state indeed seems to be suitable and sufficient for the transportation of live fish. All the carp were maintained in the anesthetized or sedate state at 8–14°C for 24 h, but the optimum range of the temperature, at which the majority of carp become sedate, is 12–14°C when the carp are acclimated at 23±1°C. Judging from the results that lower temperatures caused greater harm to fish, 14°C is the most suitable condition regardless of the anesthetic time. According to Moffitt and Crawshaw,[18] the oxygen consumption of carp kept at 14°C reduced to about 50 percent of that at 23°C. We demonstrated that the carp, survived the first cold-anesthesia and second cold-sedation for a total of 24 h. Such combination (*e.g.* 4°C and 14°C) will be useful for a short-term transfer to a carriage tank and a long-term transportation of live fish.

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