

コイ (Cyprinus carpio) の安静時における血液と呼吸循環の特性

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Blood and Cardiovascular-Respiratory Properties of the Common Carp *Cyprinus carpio* under Resting Conditions

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Abstract : We investigated the variations and the interrelationships among heart rate, blood pressure, ventilation frequency, blood gases, electrolytes, and catecholamine concentrations in the common carp *Cyprinus carpio* under resting conditions. The mean values of blood parameters and cardiovascular-respiratory properties were not significantly different between males and females. No significant sexual differences in correlation coefficients were observed. Therefore, the sexual difference hardly affected these properties of the common carp, which had immature gonad, under resting conditions. Blood Na⁺ and Cl⁻ concentrations and pH of the common carp were strictly controlled under resting conditions since the coefficient variations of Na⁺, Cl⁻ and pH were much lower than those of the other properties. Some blood properties of the resting carp were found to correlate with each other: heart rate, dorsal aortic blood pressure, ventilation frequency, blood pH, O₂ partial pressure, and catecholamine concentrations. The variations in blood pH, O₂ partial pressure, and catecholamines of the resting carp little effect on the heart rate and the blood pressure.

Key words : *Cyprinus carpio* ; resting condition; blood gas and electrolyte ; catecholamine ; heart rate ; blood pressure

Introduction

The Cyprinidae is the largest family of freshwater fish, and the cyprinids are the most diverse in Southeast Asia, followed by Africa, North America, and Europe.¹⁾ The common carp *Cyprinus carpio* is widely distributed in Europe and Asia, and is cultured for food and as an ornamental species. The common carp has been studied to clarify its respiration physiology and aquaculture environment in terms of metabolism and the oxygen transport of blood at normoxia, hypoxia, hyperoxia, hypercapnia, and exercise.²⁻¹⁰⁾ However, there are few reports on the relationship between blood and cardiovascular-respiratory properties of the common

carp under resting conditions. Therefore, we measured arterial blood gases, blood chemical properties, ventilation frequency, heart rate, and dorsal aortic blood pressure, and investigated the variations and the interrelationships of blood parameters and cardiovascular-respiratory properties of the resting carp.

Materials and Methods

Experimental animals and preparation

Common carp were obtained from a commercial farm, and reared in 500 l indoor tanks for one month at 25 °C (light/dark; 14 h/10 h). Carp were fed commercial pellets (No. 7P, Nippon formula feed Co., Ltd.) at a satiation

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quantity every day, and were fasted for 24 hr prior to the measurement of blood and cardiovascular-respiratory properties. Experimental animals were anesthetized with a 0.2 g/l solution (25 °C) of ethyl-*m*-aminobenzoate methanesulfonate (MS-222, Crescent Research Chemicals), which was neutralized with NaHCO₃ (Nacalai Tesque, Inc.). Polyethylene tubing (outer diameter 1.0 mm, No.3, Hibiki) was cannulated into the dorsal aorta, and another tube (outer diameter 1.4 mm, No.4, Hibiki) was implanted into the buccal cavity.¹¹⁾ Animals were then transferred to an acrylic darkened respiratory chamber after surgery and remained there for 24 hr. The qualities of the freshwater used for this experiment were as follows: temperature, 25 ± 0.1 °C (mean ± SD); pH, 7.60 ± 0.20; O₂ partial pressure, 150 ± 2.1 mmHg (oxygen saturation 98.7%). The mean body weight and length of experimental animals were 654 g and 299 mm (N=92). The condition factor of the experimental animals was calculated in the formula: Condition factor = body weight (kg) · body length (m)⁻³. The sex of carp was discriminated by the observation of the gonad.

Blood sampling and measurement of blood properties

Blood samples (0.1%/kg body weight) were drawn through the dorsal aortic cannula with a glass syringe in which the dead space was filled with saline (0.9% NaCl) containing heparin lithium salt (100 I.U., Sigma Chemical Co.). Arterial blood pH (pHa) and O₂ partial pressure (Pao₂, mmHg) were immediately measured with an acid-base analyzer (PHM-73, Radiometer) and blood microsystem (BMS3-Mk2, Radiometer). The arterial blood O₂ content (Cao₂, ml/100 ml) and total CO₂ concentration (Taco₂, mM/l) were measured by O₂ and CO₂ electrodes.^{12,13)} Arterial blood O₂ saturation (Sao₂, %) was calculated from Pao₂, Cao₂, the oxygen solubility coefficient, and oxygen capacity of hemoglobin. The oxygen solubility coefficient and oxygen capacity were those used in a previous study.¹⁴⁾ Arterial blood CO₂ partial pressure (Paco₂, mmHg) and bicarbonate concentration (HCO₃⁻, mM/l) were calculated using the Henderson-Hasselbalch equation.¹⁵⁻¹⁷⁾ The hematocrit values of arterial blood (Hta, %) were measured using the microhematocrit method. Hemoglobin (Hba, g/100 ml), plasma calcium (Ca²⁺, mEq/l), and plasma total protein (Tpro, g/100 ml) concentrations were determined with an auto colorimetric analyzer (DRI-CHEM 5500, Fuji Medical Systems). Plasma sodium, potassium, chloride,

calcium ions (Na⁺, K⁺, Cl⁻, Ca²⁺, mEq/l) were measured with the auto analyzer (DRI-CHEM 800, 5000, Fuji Medical Systems). Plasma magnesium concentrations (Mg²⁺, mEq/l) were determined with a commercial kit (Magnesium B-test kit, Wako Pure Chemical Industries Co.) and a spectrophotometer (Spectronic 20A, Shimadzu). Plasma noradrenaline and adrenaline (NOR, ADR, nM/l) levels were determined with alumina-extracted samples using high performance liquid chromatography and an electrochemical detector.¹¹⁾

Cardiovascular-respiratory properties measurement

The cannulae from the dorsal aorta and buccal cavity were each connected to pressure transducers (MPU-0.5-290, Nihon Koden). Dorsal aortic blood pressure (systolic pressure: BPS; diastolic pressure: BPD, mmHg) and buccal cavity pressure were recorded with an amplifier (AP-601 G, Nihon Koden) and recorder (WT-625 G, Nihon Koden). Heart rate (HR, beats/min) and ventilation frequency (Vf, cycles/min) were estimated by fluctuations in dorsal aortic blood pressure and buccal pressure¹¹⁾, respectively.

Statistical analysis

Significant differences in the mean values of each property between males and females were analyzed in the two-sample-*t*-test. After the correlation coefficients were calculated, the significance of each correlation coefficient was analyzed in the *t*-test, and the difference in the correlation coefficients of males and females was analyzed by the *z*-transform. The level of significance was *P*<0.01.

Results

The gonads of both male and female animals were undeveloped. The total length, body length, body weight, and condition factor in male and female common carp used for the experiment are shown in Table 1. There were no significant differences in the size and condition factor between males and females. The mean values of blood parameters and cardiovascular-respiratory properties are shown in Tables 2-5. There were no significant differences in these values between males and females. The coefficient variations (CV) of pHa, Na⁺, and Cl⁻ were 0.1%-0.7%, 2.4%-2.7%, and 4.1%-4.2%, respectively, which were much lower than those of other blood parameters

and cardiovascular-respiratory properties. Some blood parameters of the resting carp were significantly correlated with each other: Hba with Hta, Cao₂, or Sao₂; Hta with Sao₂ or Cao₂; and Na⁺ with Cl⁻ (Figs. 1-4), and sample regression slopes, intercepts, and correlation coefficients

are shown in Table 6. The other correlation coefficients of blood parameters and cardiovascular-respiratory properties showed no significant differences. In the correlation coefficients, there were no significant differences between males and females.

Table 1. The mean value, standard error (SE), number of individuals (N), and coefficient of variation (CV) for the size of male and female experimental animals

| | | Male | | | | Female | | | |
|------------------|------|------|------|----|------|--------|------|----|------|
| | | Mean | SE | N | CV | Mean | SE | N | CV |
| Total length | (mm) | 366 | 3.7 | 49 | 7.1 | 366 | 3.5 | 43 | 6.3 |
| Body length | (mm) | 299 | 3.3 | 49 | 7.7 | 298 | 3.4 | 43 | 7.4 |
| Body weight | (g) | 649 | 16.3 | 49 | 17.6 | 658 | 16.9 | 43 | 16.9 |
| Condition factor | | 24 | 0.3 | 49 | 8.3 | 25 | 0.5 | 43 | 12.0 |

Table 2. The mean value, standard error (SE), number of individuals (N), and coefficient of variation (CV) for the heart rate, dorsal aortic blood pressure, and ventilation frequency of male carp under resting conditions

| | | Mean | SE | N | CV |
|--------------------------|------------|------|------|----|------|
| Heart rate | beats/min | 32.0 | 1.0 | 42 | 20.0 |
| Systolic blood pressure | mmHg | 26.7 | 0.50 | 30 | 13.9 |
| Diastolic blood pressure | mmHg | 17.1 | 0.80 | 30 | 25.7 |
| Ventilation frequency | cycles/min | 13.0 | 0.90 | 29 | 34.8 |

Table 3. The mean value, standard error (SE), number of individuals (N), and coefficient of variation (CV) for the heart rate, dorsal aortic blood pressure, and ventilation frequency of female carp under resting conditions

| | | Mean | SE | N | CV |
|--------------------------|------------|------|------|----|------|
| Heart rate | beats/min | 31.0 | 0.90 | 38 | 18.6 |
| Systolic blood pressure | mmHg | 25.8 | 0.80 | 31 | 16.7 |
| Diastolic blood pressure | mmHg | 16.4 | 0.60 | 31 | 22.0 |
| Ventilation frequency | cycles/min | 14.0 | 0.90 | 29 | 32.2 |

Table 4. The mean value, standard error (SE), number of individuals (N), and coefficient of variation (CV) for the arterial blood properties ¹ of male carp under resting conditions

| | | Mean | SE | N | CV |
|-------------------------------|----------|-------|--------|----|------|
| Hta | % | 24.3 | 0.44 | 49 | 12.8 |
| Hba | g/100ml | 8.0 | 0.20 | 49 | 17.5 |
| Cao ₂ | ml/100ml | 8.1 | 0.15 | 35 | 11.1 |
| Pao ₂ | mmHg | 30.0 | 0.74 | 49 | 17.3 |
| Sao ₂ | % | 74.9 | 1.34 | 35 | 10.5 |
| pHa | | 7.779 | 0.0076 | 49 | 0.7 |
| Taco ₂ | mM/l | 10.4 | 0.22 | 34 | 12.5 |
| Paco ₂ | mmHg | 4.0 | 0.10 | 34 | 15.0 |
| HCO ₃ ⁻ | mM/l | 10.2 | 0.21 | 34 | 12.2 |
| NOR | nM/l | 2.4 | 0.20 | 25 | 41.7 |
| ADR | nM/l | 1.7 | 0.14 | 25 | 41.2 |
| Tpro | g/100ml | 2.7 | 0.11 | 21 | 18.5 |
| Na ⁺ | mEq/l | 134.0 | 0.56 | 42 | 2.7 |
| K ⁺ | mEq/l | 3.2 | 0.08 | 42 | 15.6 |
| Cl ⁻ | mEq/l | 111.0 | 0.69 | 42 | 4.1 |
| Ca ²⁺ | mEq/l | 4.3 | 0.11 | 38 | 15.6 |
| Mg ²⁺ | mEq/l | 1.7 | 0.07 | 19 | 17.6 |

¹ Hta, hematocrit value; Hba, hemoglobin concentration; Cao₂, O₂ content; Pao₂, O₂ partial pressure; Sao₂, O₂ saturation; pHa, pH; Taco₂, total CO₂ concentration; Paco₂, CO₂ partial pressure; NOR, noradrenaline concentration; ADR, adrenaline concentration; Tpro, total protein concentration.

Table 5. The mean value, standard error (SE), number of individuals (N), and coefficient of variation (CV) for the arterial blood properties ¹ of female carp under resting conditions

| | | Mean | SE | N | CV |
|-------------------------------|----------|-------|--------|----|------|
| Hta | % | 24.8 | 0.58 | 43 | 15.3 |
| Hba | g/100ml | 8.0 | 0.21 | 43 | 17.5 |
| Cao ₂ | ml/100ml | 8.1 | 0.16 | 32 | 11.1 |
| Pao ₂ | mmHg | 30.8 | 0.95 | 43 | 20.1 |
| Sao ₂ | % | 73.5 | 0.97 | 67 | 10.8 |
| pHa | | 7.776 | 0.0011 | 43 | 0.1 |
| Taco ₂ | mM/l | 10.5 | 0.27 | 30 | 14.3 |
| Paco ₂ | mmHg | 4.0 | 0.13 | 30 | 17.5 |
| HCO ₃ ⁻ | mM/l | 10.3 | 0.26 | 30 | 13.9 |
| NOR | nM/l | 2.2 | 0.16 | 24 | 36.4 |
| ADR | nM/l | 1.7 | 0.14 | 24 | 41.2 |
| Tpro | g/100ml | 2.7 | 0.10 | 17 | 14.8 |
| Na ⁺ | mEq/l | 135.0 | 0.58 | 32 | 2.4 |
| K ⁺ | mEq/l | 3.1 | 0.11 | 32 | 19.4 |
| Cl ⁻ | mEq/l | 112.0 | 0.83 | 32 | 4.2 |
| Ca ²⁺ | mEq/l | 4.6 | 0.12 | 30 | 14.1 |
| Mg ²⁺ | mEq/l | 2.0 | 0.24 | 14 | 45.0 |

¹ Hta, hematocrit value; Hba, hemoglobin concentration; Cao₂, O₂ content; Pao₂, O₂ partial pressure; Sao₂, O₂ saturation; pHa, pH; Taco₂, total CO₂ concentration; Paco₂, CO₂ partial pressure; NOR, noradrenaline concentration; ADR, adrenaline concentration; Tpro, total protein concentration.

Table 6. Sample regression slopes and intercepts of the arterial blood properties¹ in the common carp under resting conditions

| Y | X | | Sample regression | | r | N |
|-------------------|-------------------|--------|-------------------|-----------|-------|----|
| | | | slope | intercept | | |
| Cao ₂ | Hba | Male | 0.589 | 3.57 | 0.697 | 35 |
| | | Female | 0.529 | 3.84 | 0.689 | 32 |
| Sao ₂ | Hba | Male | -4.470 | 109.62 | 0.624 | 35 |
| | | Female | -4.185 | 105.50 | 0.617 | 32 |
| Paco ₂ | pHa | Male | -5.344 | 45.58 | 0.440 | 34 |
| | | Female | -9.053 | 74.44 | 0.617 | 30 |
| Paco ₂ | Taco ₂ | Male | 0.327 | 0.58 | 0.701 | 34 |
| | | Female | 0.341 | 0.43 | 0.740 | 30 |
| Cl ⁻ | Na ⁺ | Male | 0.869 | -5.85 | 0.691 | 42 |
| | | Female | 0.877 | -6.81 | 0.615 | 32 |
| Hba | Hta | Male | 1.506 | 12.26 | 0.686 | 49 |
| | | Female | 1.933 | 9.23 | 0.738 | 43 |
| Cao ₂ | Hta | Male | 0.313 | 0.39 | 0.686 | 35 |
| | | Female | 0.282 | 3.16 | 0.738 | 32 |

¹ Cao₂, O₂ content (ml/100ml); Hba, hemoglobin concentration (g/100ml); Sao₂, O₂ saturation (%); Paco₂, CO₂ partial pressure (mmHg); pHa, pH; Taco₂, total CO₂ concentration (mM/l); Hta, hematocrit value (%).

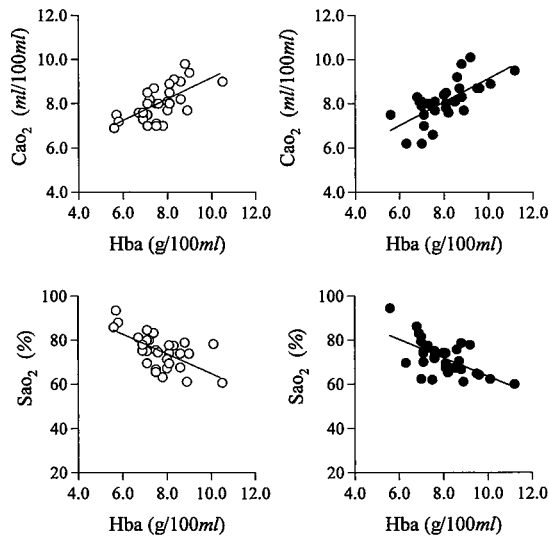


Fig.1. The relationship of arterial blood properties of the common carp under resting conditions in males (open circles) and females (closed circle): Hba, hemoglobin concentration; Cao₂, oxygen content; Sao₂, oxygen saturation. Each correlation coefficient was significant ($P < 0.01$). There are no significant differences in the correlation coefficients between males and females. The straight lines in the figures are regression line. The sample regression slopes, intercepts and correlation coefficients are shown in Table 6.

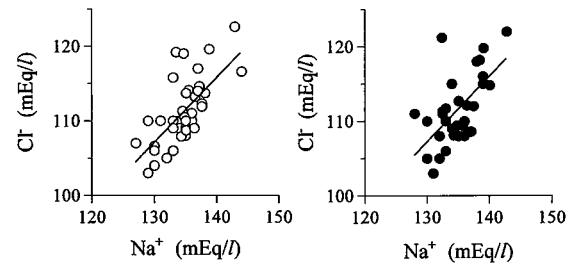


Fig.3. The relationship of Na⁺ and Cl⁻ concentrations of the arterial blood of the common carp under resting conditions in males (open circles) and females (closed circle). Each correlation coefficient was significant ($P < 0.01$). There are no significant differences in the correlation coefficients between males and females. The straight lines in the figures are regression line. The sample regression slopes, intercepts and correlation coefficients are shown in Table 6.

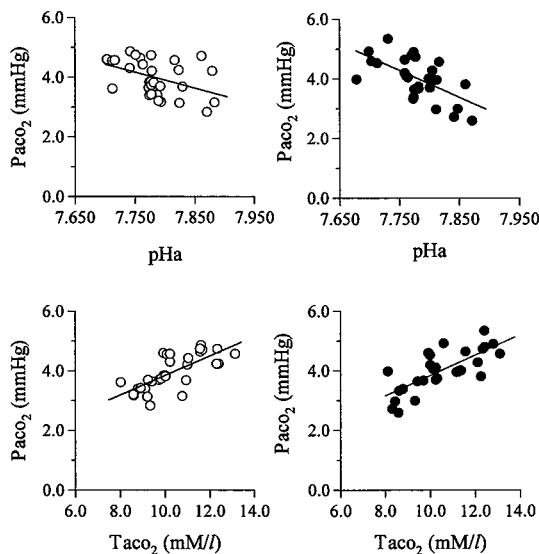


Fig.2. The relationship of arterial blood properties of the common carp under resting conditions in males (open circles) and females (closed circle): pHa, arterial blood pH; Paco₂, CO₂ partial pressure; Taco₂, total CO₂ concentration. Each correlation coefficient was significant ($P < 0.01$). There are no significant differences in the correlation coefficients between males and females. The straight lines in the figures are regression line. The sample regression slopes, intercepts and correlation coefficients are shown in Table 6.

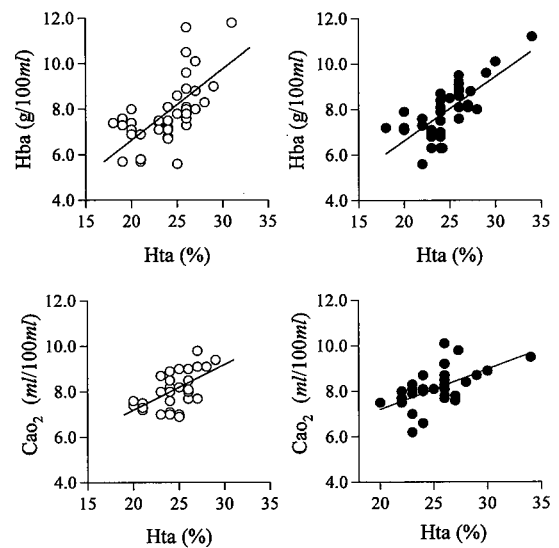


Fig.4. The relationship of arterial blood properties of the common carp under resting conditions in males (open circles) and females (closed circle): Hta, hematocrit value; Hba, hemoglobin concentration; Cao₂, oxygen content. Each correlation coefficient was significant ($P < 0.01$). There are no significant differences in the correlation coefficients between males and females. The straight lines in the figures are regression line. The sample regression slopes, intercepts and correlation coefficients are shown in Table 6.

Discussion

We investigated the variations and the interrelationships of blood parameters and cardiovascular-respiratory properties of the common carp under resting conditions. The mean values of blood parameters and cardiovascular-respiratory properties were not significantly different between males and females. No significant sexual differences in the correlation coefficient were found. Therefore, the sexual difference hardly affected the heart rate, blood pressure, ventilation frequency, blood gases, electrolytes, and catecholamine concentrations of the common carp, which had immature gonad, under resting conditions. The CV of pHa, Na⁺, and Cl⁻ were much smaller than those of other properties. It is known that pH, Na⁺, and Cl⁻ are important factors in determining O₂/CO₂ transportation and osmotic pressure.¹⁸⁾ In O₂/CO₂ transportation, the hydrogen ion is buffered by the complex buffer system of bicarbonate, protein, phosphate, and ammonia. Moreover, the function of the erythrocyte is maintained by the regulation of pH. In CO₂ transportation, a chloride shift is induced by the compartmental fluxes of Cl⁻ with the antiporter of the erythrocyte membrane. The chloride cell of the gill actively takes in Na⁺ and Cl⁻. Therefore, plasma Na⁺, Cl⁻, and blood pH of the common carp were strictly controlled under resting conditions.

Pao₂ and Sao₂ in this study were in accordance with those previously reported in *C. carpio* (Po₂ = 24-26 mmHg, Sao₂ = 68-80 %).^{2,4,6,9)} However, Cao₂ in this study was 1.3-1.5 times higher than those reported (5-7 ml/100 ml). This has been mainly attributed to the finding that Hba in this study was also 1.3-1.5 times higher than those reported (5-6 g /100 ml). The significant negative correlation between Hba and Sao₂ (Fig.1, Table 6) indicated that higher Hba blood, even with low So₂, allowed for enough oxygen to be transported for resting metabolism. The calculated Paco₂ was in accordance with carp blood Pco₂, which was directly measured (Pco₂=3.7-4.5 mmHg).^{2,4,6,9)} Thus, the calculated value in this study was equivalent to directly measured blood Pco₂. Although the total blood CO₂ concentration of the common carp (Taco₂, 10.4-10.5 mM/l) is 48%-49% that of humans (Tco₂=21.5 mM/l)¹⁹⁾, the blood CO₂ partial pressure of the common carp (Pco₂=4 mmHg) is 10% that of humans (Pco₂=40 mmHg).¹⁹⁾ These findings suggest that

the arterial blood of the common carp has an effective buffer function and that CO₂ is excreted rapidly from blood, which enables the common carp to live in ambient water where pH and dissolved gases often vary. The pH and Pco₂ of blood have a great influence on blood oxygen saturation and oxygen affinity.¹⁸⁾ Under resting conditions, Hba, Cao₂, and Sao₂ were not significantly correlated to pHa and Paco₂. Therefore, the arterial blood of common carp at rest was not affected by the "Bohr effect". This suggests that blood pH and CO₂ partial pressure had little effect on the oxygen affinity of erythrocytes within the range of blood O₂ partial pressure at rest.

NOR and ADR in this study (1.7-2.4 nM/l) were in agreement with the values previously reported in *C. carpio* (0.5-3.0 nM/l (0.1-0.5 ng/ml)⁸⁾; 0.29-14.6 nM/l (0.054-2.47 ng/ml)²⁰⁾), and with those in other teleosts (1.5-5.0 nM/l).⁸⁾ The oxygen affinity of erythrocytes increases with rising plasma catecholamine (noradrenaline, adrenaline) levels in *Oncorhynchus mykiss*²¹⁻²⁴⁾ and *Anguilla rostrata*.²⁴⁾ In this study, there were no significant correlations among NOR, ADR, Cao₂, and Sao₂. Therefore, NOR and ADR under resting conditions probably had little effect on the oxygen affinity of erythrocytes within the range of variations in plasma catecholamines at rest. In brief, the *in vivo* arterial blood of carp seemed not to be affected by the "Bohr effect" due to pHa and Paco₂ under resting conditions and arterial blood oxygen affinity did not appear to be influenced by NOR or ADR under these conditions. Increases in plasma catecholamine concentrations enhance cardiac function (*Anguilla australis*, *A. dieffenbachia*²⁵⁾; *Hemibarbus asotus*, *Macrozoarces americanus*²⁶⁾; *O. mykiss*²⁷⁻²⁸⁾). There were no significant relationships among HR, BPS, BPD, NOR, ADR, pHa and Pao₂. This suggests that blood pHa, Pao₂, NOR, and ADR probably had little effect on the heart rate and blood pressure of carp at rest. Therefore, the cardiac function of intact carp did not seem to be affected by blood gases and catecholamines within the resting variations in these properties.

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コイ (*Cyprinus carpio*) の安静時における 血液と呼吸循環の特性

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安静状態におけるコイの血液ガス、電解質、カテコールアミン濃度、心拍数、血圧、呼吸数を測定し(水温 25℃、酸素分圧 150 mmHg)、それらの相互関係について検討した。その結果、各性状はコイの雌雄間において統計的有意差が認められなかった。また、各性状間の相関係数は雌雄間で有意差は無かった。コイ血液の pH、ナトリウムイオン濃度およびクロライドイオン濃度は厳密に調整されており、それらの変動が安静状態の範囲内であれば、心拍数、血圧および呼吸数に有意な影響を与えない事が明らかとなった。さらに、血液の酸素分圧、酸素含量、二酸化炭素分圧およびカテコールアミン濃度の変動が安静状態の範囲内にあれば、心拍数と血圧および呼吸数に対して顕著な影響を及ぼさない事が明らかとなった。