

Ventilation of the Air-breathing Organ in the Snake-head *Channa argus*

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Abstract *Channa argus* breathes air in the following manner: The opercula begin to abduct before the fish breaks the surface. The buccal cavity distends to transport gas from the air-breathing organ into the cavity. Expiration seems to be effected by a hydrostatic head and the contraction of the buccal and pharyngeal cavities, and is done directly into the air. Then the buccal cavity again distends to inspire air. The following abduction of the opercula transports the gas into the air-breathing organ while the branchiostegal membrane prevents the backflow of water. When the fish is breathing bimodally in water, nearly complete renewal of gas occurs at each air breath as evidenced by close agreement between composition of the gas in the air-breathing organ just after inspiration and that of the atmospheric air. When the fish is deprived from water, it violently struggles and repeats air breathing at a markedly higher rate. Ventilation of the air-breathing organ becomes less efficient in this condition, which is illustrated by lower P_{O_2} and higher P_{CO_2} of the gas throughout a breath hold. Our conclusion is that the gas in the organ is replaced by water entering the organ during expiration and then the water is replaced by inspired air when the fish breathes air during bimodal breathing.

Fish show a wide variety of ventilatory mechanisms ranging from continuous flow of water across the gills to tidal ventilation of air (Hughes, 1978). Among air-breathing fishes, one can also notice many types of ventilation as is expected from anatomical diversity of their air-breathing organs (Abdel Magid, 1966; Bishop and Foxon, 1968; McMahon, 1969; Rahn et al., 1971; Graham et al., 1977; Farrell and Randall, 1978; Kramer, 1978; Peters, 1978).

Channa argus (Cantor) has a pair of specialized air-breathing organs (suprabranchial organ) with which 60% of O_2 uptake and 15% of CO_2 release are satisfied in well aerated water at 25°C (Itazawa and Ishimatsu, in preparation). The circulatory system of *C. argus* (and *C. maculata*) was studied previously (Ishimatsu et al., 1979). Air-breathing organs of fish function mainly for O_2 uptake and most of the CO_2 produced is released by the gills. As a result, surfacing of these animals appears to need much less time than in mammals where CO_2 retention is inevitable during diving (Rahn and Howell, 1976).

In the present study we attempted to observe how air breathing is carried out in *C. argus* and to assess its efficiency by analyzing the gas in the air-breathing organ.

Material and methods

Eight specimens of *C. argus* were collected on hook and line in Fukuoka and Saga Prefectures. The fish were maintained in the laboratory as described elsewhere (Itazawa and Ishimatsu, in preparation).

Air breathing behavior. One specimen weighing 385 g was filmed at 24 frames/sec with an ordinary 8 mm cine-camera. Some thirty films were made, ten each of dorsal, lateral and ventral views, and fifteen, five of each, were analyzed thereafter. For making films in the latter two views, the fish was confined in a small plexiglass box (60 × 13 × 10 cm) held at an angle of 30°, the angle being determined by preliminary observations. Because the films were not simultaneously made, the most definite stage (stage e, see later) was taken as time 0 and then the time intervals between this and other stages were determined by counting frame numbers.

Composition of the gas in the air-breathing organ. To know the time course of the changes in composition of the gas in the air-breathing organ, gas samplings were made on seven specimens (500~800 g in body weight) when they breathed bimodally in well aerated water ($P_{w,O_2} \geq 130$ mmHg), and on five when they breathed

in air. The fish were anesthetized by immersing them in a solution of quinaldine in concentration of 1:10000. A polyethylene catheter was

implanted into the air-breathing organ. The fish were then allowed to recover for 24 hr in a large container through which well aerated water

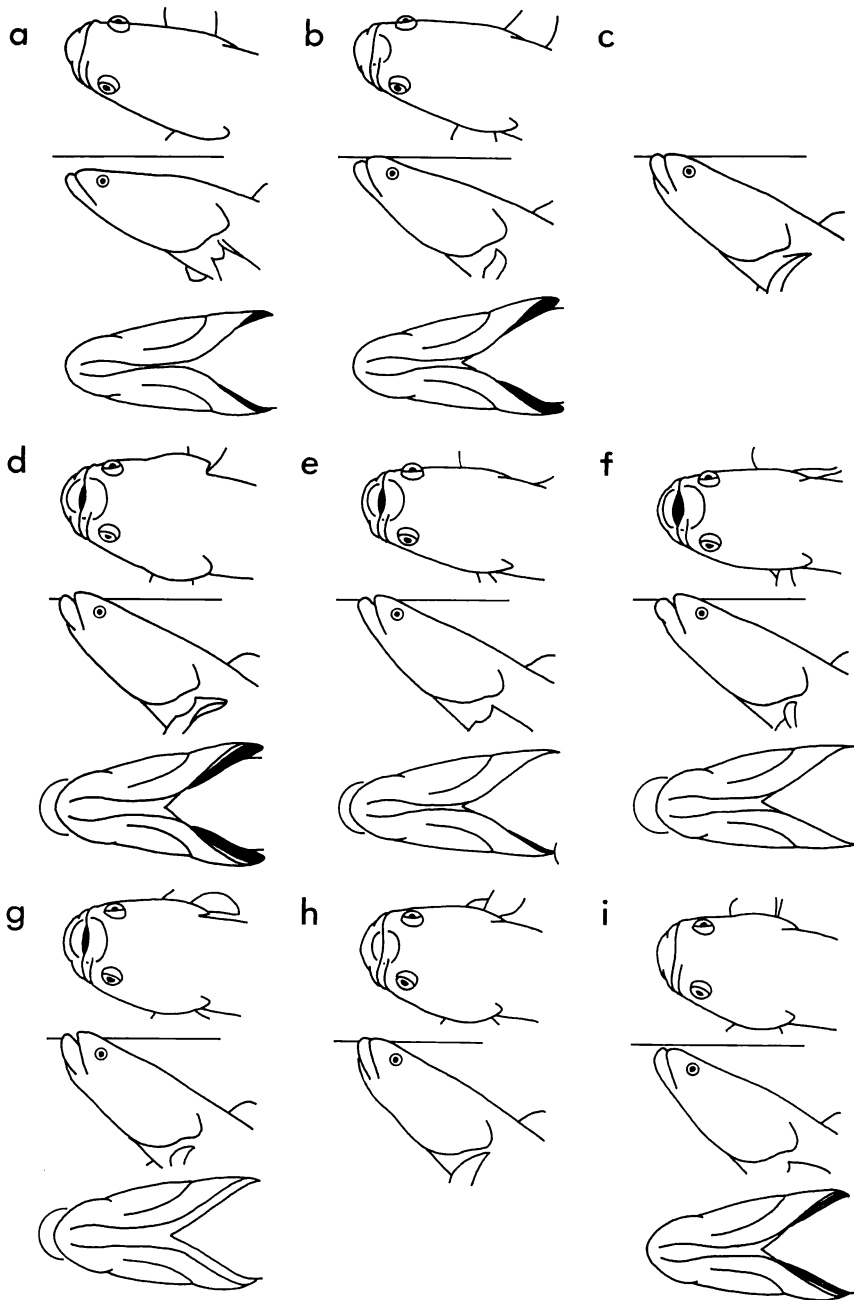


Fig. 1. Sequence of air breathing in *Channa*. The tracings were made from film recordings of dorsal, lateral and ventral views taken at an exposure rate of 24 frames/sec. Stages a~i correspond to those in Table 1.

Table 1. Timing of air breathing sequence in *Channa*. Values are expressed in mean ± 1 SD. Numbers in parentheses show numbers of observations. Stage a~d represents preparation for expiration, d~e is expiration, and f~h is inspiration.

Stage	Time (sec)
a Opercula begin to expand	-1.90 ± 0.45 (5)
b Snout touches the surface	-1.22 ± 0.45 (10)
c Buccal floor is depressed	
d Mouth opens	-0.18 ± 0.03 (10)
e Buccal floor is raised	0
f Mouth opens more widely	0.04 ± 0.00 (10)
g Opercula expand with the clefts closed	
h Mouth closes	0.18 ± 0.02 (10)
i Snout leaves the surface	0.41 ± 0.06 (10)

was gently circulated. Water in the container was drained in order to obtain gas samples from air-exposed fish. A thin film of water at the bottom of the container prevented the fish from drying during air-exposure of 5 hr. Gas sampling was made only once in a breath hold, because it reduced gas volume in the organ and

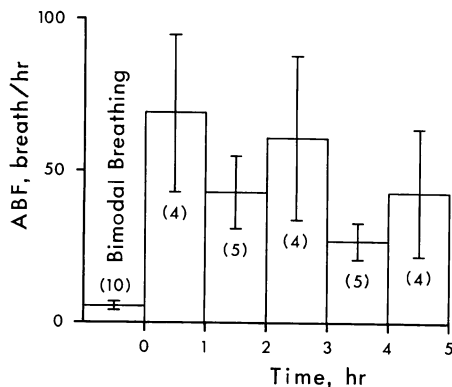


Fig. 2. Air breathing frequency (ABF) observed during 5 hr air-exposure at 25°C. The leftmost column shows ABF when *Channa* breathes bimodally in well aerated water ($P_{w,O_2} \geq 130$ mmHg). The numbers of observations are shown in parentheses. The vertical bars represent ± 1 SD.

possibly affected gas composition. Gas samples were analyzed for O_2 and CO_2 concentrations with a Scholander micro gas analyzer.

Results

Air breathing behavior. When *C. argus* breathes air, it approaches the surface at angles of 30~40°. The opercula begin to abduct and the head is raised while the fish is ascending to the surface (Fig. 1, stage a). The opercula continue to abduct after the snout touches the surface, and the opercular clefts are widely opened. The buccal cavity begins to expand following the abduction of the opercula (stage b). Just before expiration, depression of the buccal floor reaches its maximum (stage c). Then the mouth is opened and expiration begins (stage d). At this stage, the opercular clefts are still opened. The buccal floor is now raised and the opercula abduct rapidly (stage e). After a short while, the mouth is opened more widely and the buccal cavity again expands with the opercula firmly attaching to the body wall (stage f). Then the opercula begin to abduct whereas the clefts are closed by extension of the branchiostegal membrane (stage g). The mouth is closed (stage h) and the fish leaves the surface (stage i). At this stage, the opercula again abduct and the opercular clefts are opened. The head leans downward and the opercula abduct completely.

Thus expiration precedes inspiration in this species. Air breathing mostly takes place only through the mouth. Exceptionally, gas bubbles are released through the opercular clefts during descent. Table 1 shows the quantitative analysis of air breathing. Both expiration and inspiration required less than 0.2 sec whereas preparation for expiration required a longer period of about 1.7 sec.

When the fish was removed from water it violently struggled in search for water. This violent reaction occurred immediately in four specimens while the other one stayed quiet for as long as 65 min. When the fish ventilates air in this condition, it raises the head above the substratum, and opens the mouth far more widely than when in water. Interrupted movement of ventilation was frequently observed. Air breathing frequency markedly increased during exposure to air (Fig. 2). Most air breathing intervals ranged from 0.5 min to

3.0 min. During breath holding of 2~3 min or longer, feeble ventilation similar to gill ventilation was usually observed.

Composition of the gas in the air-breathing organ. Fig. 3 shows the time course of the changes in composition of the gas in the air-breathing organ. When the fish was in water both P_{O_2} and P_{CO_2} approached the atmospheric level just after inspiration ($P_{O_2}=146.9\pm 2.7$ mmHg and $P_{CO_2}=3.7\pm 2.0$ mmHg, $N=7$). These values are practically identical with those of the atmosphere ($P_{O_2}\doteq 155$ mmHg and $P_{CO_2}\doteq 0$ mmHg), when the time lag between inspiration and actual sampling time (ca. 10 sec) is taken into consideration. Then P_{O_2} decreased continuously down to 62.6 ± 11.0 mmHg ($N=5$) in 10 min of breath holding. P_{CO_2} increased only in the first 2~3 min and then remained on a steady level at 21 mmHg. The gas exchange ratio for the air-breathing organ (calculated by J_{PCO_2}/J_{PO_2}) was 0.5~0.8 in the early phase of breath holding, but it declined to zero after 3 min of breath holding. The overall gas exchange ratio for 10 min breath holding was about 0.2.

During exposure to air, P_{CO_2} of the gas in the organ was consistently higher than values obtained when the fish was in water. Initial P_{CO_2} of 16.4 ± 5.4 mmHg ($N=5$) further increased to 27.0 ± 2.3 mmHg ($N=4$) in 3 min of breath holding. P_{O_2} varied considerably, but it was apparently below the atmospheric level even immediately after inspiration (119.8 ± 28.2 mmHg, $N=5$). The gas exchange ratio reached 0.7 in the first 1 min of breath holding, but it precipitously fell to 0.2 in the next two minutes.

Discussion

The present observations allow us to delineate the possible mechanism of air breathing in *C. argus*. Interpretation of the observations is as follows: Air breathing starts with the abduction of the opercula. Since the opercular clefts are opened and the fish is in an oblique position to the surface, water must enter the air-breathing organ (Fig. 1, stages a and b). The organ is divided by the hyomandibular process which projects from the inner wall of the operculum (Fig. 4) so that water first fills the subcavity posterior to the process and then flows into the anterior subcavity. The gas is further transported anteriorly by the distention of the buccal

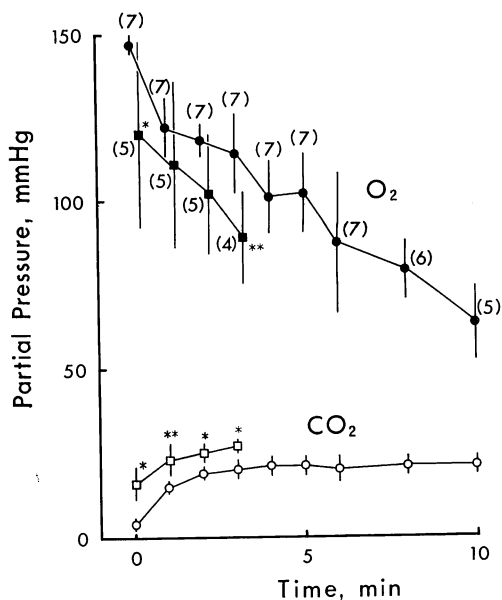


Fig. 3. Time course of the changes in composition of the gas in the air-breathing organ when *Channa* breathes bimodally in well aerated water (\bullet P_{O_2} , \circ P_{CO_2}), and when deprived from water (\blacksquare P_{O_2} , \square P_{CO_2}). Mean \pm 1 SD. The numbers of fish used are shown in parentheses. The numbers for CO_2 points are same as corresponding O_2 points. Symbols with asterisk(s) are statistically different from corresponding values obtained from bimodally breathing fish (* $p < 0.01$, ** $p < 0.05$; Mann-Whitney U test).

cavity (Fig. 1, stage c). Expiration may be accomplished by a hydrostatic head with the assistance of the contraction of the buccal musculature and the abduction of the opercula (Fig. 1, stage e). Close agreement between composition of the gas in the air-breathing organ just after inspiration and that of the atmosphere (Fig. 3) provides evidence that the organ is almost completely emptied before inspiration. Inspiration must be an active process and buccal suctioning is responsible for it (Fig. 1, stage f). The following abduction of the opercula is interpreted as a suctioning of the inspired gas into the air-breathing organ (Fig. 1, stage g). The branchiostegal membrane closes the opercular clefts and prevents the backflow of water. The opercula then abduct and the water contained in

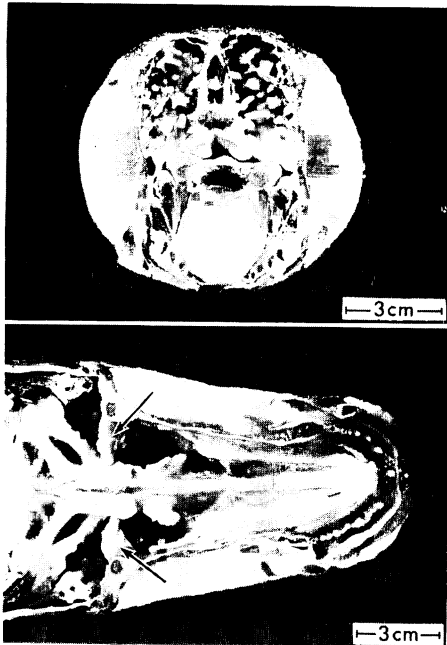


Fig. 4. Cross (upper) and sagittal (lower) sections of the head of *Channa* showing the air-breathing organ (suprabranchial organ). Cross sectioning was made just anterior to the hyomandibular process (arrow). Left hyomandibular process in cross section was partly cut so that the posterior subcavity of the air-breathing organ can be seen in the dorsal margin.

the buccopharyngeal cavity is expelled (Fig. 1, stage i). The whole sequence is quite similar to the diphasic ventilation of anabantoids observed by Peters (1978).

Munshi (1962) speculated about the breathing mechanism of *Ophicephalus* (= *Channa punctatus*) based on anatomical investigation. He claimed that expiration takes place through the opercular clefts, and is brought about by contraction of the pharynx and the suprabranchial organ. His statement is incompatible with our observations. We rarely noticed gas bubbles leaving through the opercular clefts. More importantly, gas analysis demonstrated that nearly complete renewal of gas occurs at each air breath. The rigid and almost uncontractile nature of the air-breathing organ excludes the possibility that such complete renewal of gas is attained simply by muscular contraction. Thus, the breathing mechanism proposed by Munshi is inapplicable

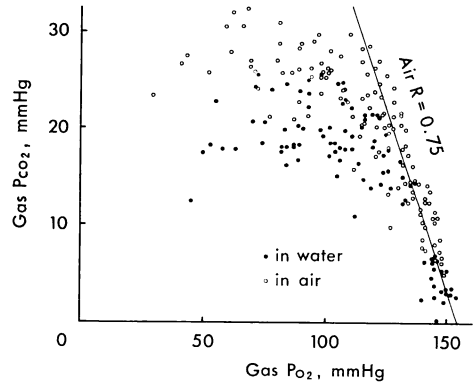


Fig. 5. PO_2 - PCO_2 diagram adapted to composition of the gas in the air-breathing organ taken from bimodally breathing fish (●) and air-exposed fish (○) with theoretical aerial gas exchange line of $R=0.75$ assuming inspired $PO_2=155$ mmHg and $PCO_2=0$ mmHg (see text).

to the congeneric species, *C. argus*. The only reasonable interpretation of the present results is, therefore, that the gas in the air-breathing organ is replaced by water entering the organ and then the water is replaced by the inspired air, as described above. That such a ventilatory mechanism actually works is also supported by the fact that ventilation becomes less functional in the absence of water, which is demonstrated by lower PO_2 and higher PCO_2 just after inspiration. It should be stated that our gas sampling method must have caused an unavoidable contamination by ambient air when it was applied to the air-exposed fish, because the air-breathing organ seems to be unable to insulate itself from the ambient air. Hence gas composition in situ in this condition must have differed more from that of air than the present results show.

Complete or nearly complete renewal of the gas in the air-breathing organ is reported to occur in other air-breathing fishes, such as several anabantoids (Peters, 1978) and *Electrophorus* (Farber and Rahn, 1970). Gas analysis of the air sac gas on two synbranchiids implies that it holds true for the two species, *Symbranchus* (Johansen, 1966) and *Amphipnous* (Lomholt and Johansen, 1976). Air-breathing organs of these animals are located at the mouth and/or the opercular cavity so that there appears to be less anatomical dead space. Among

fishes which utilize the gas bladder or lung as an air-breathing organ, tidal volume ranges widely, that is, 14% of the volume of the air-breathing organ in *Piabucina* (Graham et al., 1977), 40% in *Lepisosteus* (Rahn et al., 1971), 60% in *Polypterus* (Abdel Magid et al., 1970), 70% in *Arapaima* (Randall et al., 1978) and 80% in juvenile *Protopterus* (Jesse et al., 1967). The large tidal volume/lung volume ratio is a characteristic of diving animals. The ratio reaches 80~90% for porpoises and whales as compared with only 16% for man (see Andersen, 1966 for review). This large renewal of gas is associated with their intermittent breathing habits.

A P_{O_2} - P_{CO_2} diagram is adapted to the data on composition of the gas in the air-breathing organ (Fig. 5). The oblique line represents the theoretical aerial gas exchange line of $R=0.75$. Points both for bimodally breathing fish and for air-exposed fish keep along with the line near the inspired point of $P_{O_2}=155$ mmHg. The data points for air-exposed fish follow the line down to P_{O_2} of about 120 mmHg. At lower P_{O_2} , P_{CO_2} levels off around 30 mmHg. As for data points concerning bimodally breathing fish, they begin to depart from the line at a higher P_{O_2} and P_{CO_2} levels off at a lower level (ca. 20 mmHg). This higher P_{CO_2} of the gas obtained in air-exposed fish indicates that the blood P_{CO_2} was elevated in this condition. Thus, CO_2 accumulated to some extent during air-exposure in spite of relatively high R value (0.75) obtained when *C. argus* breathed in air (Itazawa and Ishimatsu, in preparation). Frequent ventilation of air observed during air-exposure (Fig. 2) may be caused by elevated blood P_{CO_2} . This CO_2 accumulation can be ascribed to the inefficiency of ventilation rather than to decreased functional gas exchange area (by collapsing of the gills) because artificial ventilation drastically decreased or even completely abolished voluntary ventilation and restored blood P_{CO_2} to its normal level (Ishimatsu and Itazawa, unpublished).

Considering the present results, we may be able to say that air breathing in *C. argus* has developed as a measure of O_2 acquisition in oxygen-poor aquatic life, but not as a preadaptation to terrestrial life, because the ventilation necessitates the existence of water. Furthermore the gas exchange organ located near the

exterior like the suprabranchial organ of *C. argus* would not prevent water loss by evaporation when the animal is deprived from water.

Literature cited

- Abdel Magid, A. M. 1966. Breathing and function of the spiracles in *Polypterus senegalus*. *Anim. Behav.*, 14: 530~533, figs. 1~4.
- Abdel Magid, A. M., Z. Vokac and Nasr El Din Ahmed. 1970. Respiratory function of the swimbladders of the primitive fish *Polypterus senegalus*. *J. Exp. Biol.*, 52: 27~37, figs. 1~4.
- Andersen, H. T. 1966. Physiological adaptations in diving vertebrates. *Physiol. Rev.*, 46: 213~243.
- Bishop, I. R. and G. E. H. Foxon. 1968. The mechanism of breathing in the South American lungfish, *Lepidosiren paradoxa*; a radiological study. *J. Zool., Lond.*, 154: 263~271, figs. 1~2, pls. 1~5.
- Farber, J. and H. Rahn. 1970. Gas exchange between air and water and the ventilation pattern in the electric eel. *Resp. Physiol.*, 9: 151~161, figs. 1~6.
- Farrell, A. P. and D. J. Randall. 1978. Air-breathing mechanics in two Amazonian teleosts, *Arapaima gigas* and *Hoplerhythrinus unitaeniatus*. *Can. J. Zool.*, 56: 939~945, figs. 1~4.
- Graham, J. B., D. L. Kramer and E. Pineda. 1977. Respiration of the air breathing fish *Piabucina festae*. *J. Comp. Physiol.*, 122: 295~310, figs. 1~8.
- Hughes, G. M. 1978. Some features of gas transfer in fish. *Bull. Inst. Maths.*, 14: 39~43, figs. 1~8.
- Ishimatsu, A., Y. Itazawa and T. Takeda. 1979. On the circulatory systems of the snakeheads *Channa maculata* and *C. argus* with reference to bimodal breathing. *Japan. J. Ichthyol.*, 26: 167~180, figs. 1~23.
- Jesse, M. J., C. Shub and A. P. Fishman. 1967. Lung and gill ventilation of the African lung fish. *Resp. Physiol.*, 3: 267~287, figs. 1~11.
- Johansen, K. 1966. Air breathing in the teleost *Symbranchus marmoratus*. *Comp. Biochem. Physiol.*, 18: 383~395, figs. 1~7.
- Kramer, D. L. 1978. Ventilation of the respiratory gas bladder in *Hoplerhythrinus unitaeniatus* (Pisces, Characoidei, Erythrinidae). *Can. J. Zool.*, 56: 931~938, figs. 1~7.
- Lomholt, J. P. and K. Johansen. 1976. Gas exchange in the amphibious fish, *Amphipnous cuchia*. *J. Comp. Physiol.*, 107: 141~157, figs. 1~9.
- McMahon, B. R. 1969. A functional analysis of the aquatic and aerial respiratory movements of an African lungfish, *Protopterus aethiopicus*, with

- reference to the evolution of the lung-ventilation mechanism in vertebrates. *J. exp. Biol.*, 51: 407~430, figs. 1~5, pls. 1~3.
- Munshi, J. S. D. 1962. On the accessory respiratory organs of *Ophicephalus punctatus* (Bloch.) and *Ophicephalus striatus* (Bloch.). *J. Linn. Soc.*, 44: 616~626, fig. 1, pls. 1~4.
- Peters, H. M. 1978. On the mechanism of air ventilation in Anabantoids (Pisces: Teleostei). *Zoomorphologie*, 89: 93~123, figs. 1~12.
- Rahn, H., K. B. Rahn, B. J. Howell, C. Gans and S. M. Tenney. 1971. Air breathing of the garfish (*Lepisosteus osseus*). *Resp. Physiol.*, 11: 285~307, figs. 1~6.
- Rahn, H. and B. J. Howell. 1976. Bimodal gas exchange. In G. M. Hughes, ed.: *Respiration of amphibious vertebrates*. Academic Press, London, xix+402 pp.
- Randall, D. J., A. P. Farrell and M. S. Haswell. 1978. Carbon dioxide excretion in the pirarucu (*Arapaima gigas*), an obligate air-breathing fish. *Can. J. Zool.*, 56: 977~982, figs. 1~4.
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Addendum

After we had prepared this manuscript, we received new information about air ventilation in *Channa punctatus* (Liem, 1980). Alternate replacement of air and water during air ventilation was observed, by cineradiography, in that species. Our conclusion is basically in harmony with his observation. However, almost complete renewal of the gas in the air-breathing organ was strongly suggested based

on gas analysis in *C. argus*, while a residual gas was observed in the organ after expiration in *C. punctatus*. (Liem, K. F. 1980. Air ventilation in advanced teleosts: Biomechanical and evolutionary aspects. In M. A. Ali, ed.: *Environmental physiology of fishes*. Plenum Press, New York, xi+723 pp.)

The paper cited as "Itazawa and Ishimatsu, in preparation" in the text has been published as follows: Itazawa, Y. and A. Ishimatsu. 1981. Gas exchange in an air-breathing fish, the snakehead *Channa argus*, in normoxic and hypoxic water and in air. *Bull. Japan. Soc. Sci. Fish.*, 47: 829~834, figs. 1~3.

カムルチーの空気呼吸行動

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カムルチーの空気呼吸行動は鰓蓋の外転により始まる。吻端が水面に到達する以前にこの外転は開始され、この時魚体は水面に対して30~40度の角度に保たれている。吻端が水面に到達した後にもこの外転運動は持続し、続いて口腔が拡張する。次いで口が開いて呼息運動が始まり、口腔が収縮し、鰓蓋が急速に内転する。次に再び口腔が拡張し、吸息運動が開始される。やや遅れて鰓蓋が外転するが、この時外鰓孔は鰓膜により閉じられている。口が閉じ、吻端が水面を離れた後鰓蓋が内転し呼吸運動が完了する。呼息および吸息運動共に約0.2秒を要した。空気呼吸器官内のガスを分析した結果、呼息に際して空気呼吸器官内のガスはほぼ完全に呼出される事が判明した。魚を空气中に置いた場合、空気呼吸頻度は顕著に上昇し、換気効率が低下した。これらの事実から、カムルチーが空気呼吸をする際には、呼息時に空気呼吸器官内のガスが外鰓孔より浸入した水によってほぼ完全に置換され、続く吸息時にこの水が吸気によって置換されているものと推測した。

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訂正・Errata

魚類学雑誌 28 卷 3 号 “石松 惇・板沢靖男：カムルチーの空気呼吸行動”の中に，編集主任（多紀）が責任を負うべき誤記がありました。著者と読者にお詫び申し上げます，下記に訂正いたします。

Errors for which the managing editor is responsible were developed in “Atsushi Ishimatsu and Yasuo Itazawa: Ventilation of the air-breathing organ in the snakehead *Channa argus*”, Japanese Journal of Ichthyology, 28 (3). The errors are corrected as follows:

Page	Line	For 誤	Should read 正
278	R19	abduct rapidly (stage e).	adduct rapidly (stage e).
278	R27	...again abduct and...	...again adduct and...
278	R29	...opercula abduct completely.	...opercula adduct completely.
279	R 4	...and the abduction of the...	...and the adduction of the...
279	R17	...then abduct and...	...then adduct and...
281	L24	...levels off around...	...levels off at around...
282	R 8	...has been publised...	...has been published...