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NOTE

# Buoyancy disorders in pet axolotls *Ambystoma mexicanum*: three cases

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ABSTRACT: As far as we are aware, there are no previous reports on the pathologic conditions of buoyancy disorders in *Ambystoma mexicanum*. Herein, we describe various clinical test results, clinical outcomes, and the pathological findings of an experimental pneumonectomy procedure in 3 *A. mexicanum* exhibiting abnormal buoyancy. The 3 pet *A. mexicanum* were adults, and their respective ages and body weights were 1, 5, and 6 yr and 48, 55, and 56 g. Two of these cases were confirmed via radiographic examination to have free air within the body cavity, and all 3 cases were found via ultrasonography to have an acoustic shadow within the body cavity and were diagnosed with pneumocoelom. Lung perforations were detected macroscopically in 2 of the cases, and all 3 cases had fibrosis in the caudal ends of the lungs. Removal of the lung lesions eliminated the abnormal buoyancy in all 3 cases. We concluded that air had leaked into the body cavity from the lungs, and we propose that lung lesions are an important cause of buoyancy disorders in *A. mexicanum*.

KEY WORDS: *Ambystoma mexicanum* · Axolotl · Buoyancy · Pneumocoelom · Pneumonectomy



## INTRODUCTION

*Ambystoma mexicanum* is a neotenic salamander classified in the family Ambystomatidae. The native habitat of *A. mexicanum* is Lake Xochimilco in Mexico City, but the native population has recently been decreasing as a result of urban development. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) protects *A. mexicanum* (Gresens 2004). Captive-bred individuals are used as research animals in fields such as embryology and endocrinology (Zhang et al. 2003, Safi et al. 2004, Kühn et al. 2005, Robles-Mendoza et al. 2011, Reiß et al. 2015). Reports on diseases in pet *A. mexicanum* include infectious diseases (Del-Pozo et al. 2011, Whited et al. 2013) and neoplastic disorders (Khudoley & Eliseiv 1979, Menger et al. 2010, Shioda et al. 2011).

Usually, clinically assessed buoyancy disorders are seen in sea turtles and aquarium fish. Such disorders are diagnosed when animals are either floating on the surface or lying on the bottom of the water (Wildgoose 2007). They generally respond poorly to treatment, and treatment methods remain a subject of investigation (Wildgoose 2007).

Buoyancy disorders are frequently encountered in *A. mexicanum* brought into veterinary practices, but since there are no academic reports on buoyancy disorders in the species, the associated pathology and pathogenesis remain unclear. Herein, we describe various clinical test results and the pathological findings of experimental pneumonectomy in 3 *A. mexicanum* axilotls exhibiting abnormal buoyancy.

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## CASE REPORTS

Three pet *Ambystoma mexicanum* exhibiting abnormal buoyancy (floating at the surface) were seen at a veterinary hospital. The respective ages and body weights of the 3 cases were 1, 5, and 6 yr and 48, 55, and 56 g. To diagnose these cases, the options of X-ray examinations (40 kV, 200 mA; exposure time: 0.025 s), gastrointestinal series (Iohexol, 5 ml $kg^{-1}$ oral gavage with sonde [18 G, 64 mm]), computed tomography (X-ray: 80 kV, 400 µA; scan time: 18 s; field-of-view: 160 mm; pixel size: 320.0 µm), ultrasonography (linear, 7.5 MHz), blood tests (packed cell volume and 15 biochemical tests: dry chemistry analyzer), and exploratory laparotomy under anesthesia (0.2% tricaine methanesulfonate) were presented to the owners, and we implemented the tests that were approved by the owners. The excised tissues were fixed in 20% formalin, embedded in paraffin, cut into 4 µm thick sections, and stained with hematoxylin and eosin, Azan mallory, Giemsa, Periodic acid-Schiff and Ziehl–Neelsen stain.

### Case 1

Case 1 was a leucistic male which had reportedly exhibited increased inhalation of air at the surface of the water since the previous day; its body had expanded and it floated on the surface. The axolotl exhibited cloacal prolapse (Fig. 1A). The animal had an appetite. Physical examination revealed body expansion and a cloacal prolapse containing gas. The owner did not consent to X-ray examinations, gastrointestinal series, or a computed tomography scan (CT), so these tests were not implemented. An acoustic shadow was detected via ultrasonography. Blood test results are shown in Table 1. When we performed a paramedian incision for an exploratory laparotomy, the gas was retained in the body cavity and the gas in the cloacal prolapse disappeared. The caudal side of the left lung was dark red, while the tip of the caudal side of the right lung was white (Fig. 1B). A macroscopic perforation site was found on the caudal side of the left lung, and the right lung was also at risk of perforation, so the affected areas of the left and right lungs were ligated and resected. The cloacal prolapse was then reduced to the point that it could be retained inside the body cavity. There were no macroscopic abnormalities found in other organs. *Citrobacter* sp. was identified via aerobic bacterial culture tests on samples from the resected lung, but the anaerobic culture tests were negative.

After awakening from anesthesia, the subject was able to maintain a normal position at the bottom of the tank. After appropriate drug susceptibility testing, enrofloxacin was administered during the stay at the veterinary hospital. Seventeen days after surgery, the axolotl resumed eating, and no recurrence was seen as of 5 mo after surgery.

Histopathological tests on the resected lung revealed the formation of lumen lined with epithelial cells ranging from cuboidal to columnar in the blind end of the right lung and hyperplasia of connective tissue around that area. Some of the epithelial cells were large but not atypical. The lumen of the left lung was lined with cuboidal ciliated epithelial cells and goblet cells, and the surrounding connective tissue was focally proliferative, similar to that noted in the right lung. There was also expansion of the capillaries, nuclear debris, and a few macrophages

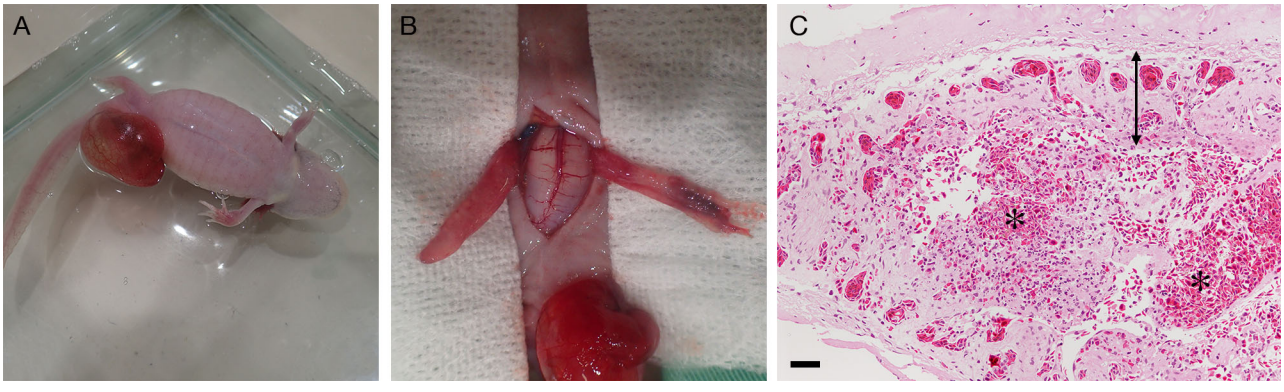


Fig. 1. *Ambystoma mexixanum* exhibiting abnormal buoyancy. Case 1. (A) Abnormal buoyancy in the water and cloacal prolapse. (B) Caudal side of the left lung is dark red; tip of the caudal side of the right lung is white. (C) Histology of the resected lungs. Fibrosis and vasodilatation present around the lesion; inflammatory cell infiltration in the center. Asterisks: hemorrhages in the lumen. Arrow: dense connective tissue and neovascularization. Scale bar = 100 µm

Table 1. Blood test results for 3 axolotls. RI: reference interval (Takami & Une 2017)

| Test | Unit | Case 1 | Case 2 | Case 3 | RI |
|---|---|---|---|---|---|
| Packed cell volume | % | 40 | 29 | 26 | 28.0–32.7 |
| Alanine aminotransferase | U $l^{-1}$ | 59 | 25 | 32 | 14.9–20.6 |
| Alkaline phosphatase | U $l^{-1}$ | 709 | 358 | 293 | 452.1–561.3 |
| Aspartate aminotransferase | U $l^{-1}$ | 210 | 153 | 193 | 201.3–263.8 |
| Blood urea nitrogen | mg $dl^{-1}$ | 6.4 | 3.2 | 5.8 | 9.9–14.5 |
| Creatinine | mg $dl^{-1}$ | 0.2 | 0.2 | 0.3 | 0.25–0.37 |
| Glucose | mg $dl^{-1}$ | 53 | 34 | 21 | 26.9–32.1 |
| Calcium | mg $dl^{-1}$ | 6.2 | 7.4 | 7.9 | 7.3–8.2 |
| Phosphorus | mg $dl^{-1}$ | 7.3 | 5.0 | 4.2 | 3.4–3.9 |
| Chloride | mmol $l^{-1}$ | 92 | 87 | 77 | 78.1–80.3 |
| Potassium | mmol $l^{-1}$ | 3.9 | 3.1 | 3.2 | 2.5–2.9 |
| Sodium | mmol $l^{-1}$ | 111 | 115 | 107 | 108.7–111.4 |
| Ammonia | µmol $dl^{-1}$ | 299 | 212 | 159 | 117.9–145.7 |
| Albumin | g $dl^{-1}$ | 0.2 | 0.3 | 0.3 | 0.44–0.51 |
| Globulin | g $dl^{-1}$ | 1.2 | 1.6 | 1.1 | 1.34–1.55 |
| Total protein | g $dl^{-1}$ | 1.4 | 1.9 | 1.4 | 1.78–2.05 |

foci heterophils in some areas. Degeneration and necrosis progressively increased caudally and was accompanied by fluid accumulation (edema) within the interstitium and free erythrocytes within the bronchus (hemorrhage). The condition was diagnosed as granulomatous pneumonia mainly in the blind end of the lung, but a pathogen was not identified (Fig. 1C).

## Case 2

Case 2 was a melanoid female which had reportedly exhibited increased inhalation of air at the surface of the water for the past 3 wk. Its body had expanded and floated on the surface. When the axolotl was brought to the veterinary hospital, it exhibited floating with the right side of the body towards the top, a state which had reportedly developed 2 d earlier (Fig. 2A). The animal still had an appetite. Physical examination indicated swelling of the trunk that exceeded the breadth of the head. Blood test results are shown in Table 1. X-ray examinations and CT showed free air within the body cavity, and an acoustic shadow was detected via ultrasonography. In a gastrointestinal series, the contrast agent was excreted 10 h after ingestion, and there was no leakage of the contrast agent within the body cavity. The cause of the free air in the body cavity was tentatively diagnosed as a leak from the lungs rather than leakage of gastrointestinal gas, so on the following day we performed an exploratory laparotomy under anesthesia. The right lung was white and adhered to the liver, and a macroscopic perforation site was found on the caudal side of the right lung (Fig. 2B). Macroscopically, the left lung appeared normal. After separating the right lung from the liver, the cephalic side of the lung was ligated and resected. No macroscopic abnormalities were detected in other organs. Both aerobic and anaerobic bacterial culture tests on the resected lung were negative. After awakening from anesthesia, the animal was able to maintain a normal position at the bottom of the tank, and after 48 h it ate some formulated pet food. The subject was discharged after 10 d, and there was no recurrence as of 5 mo after surgery. Histopathological tests on the resected lung revealed that the lung-specific tissue had been replaced by

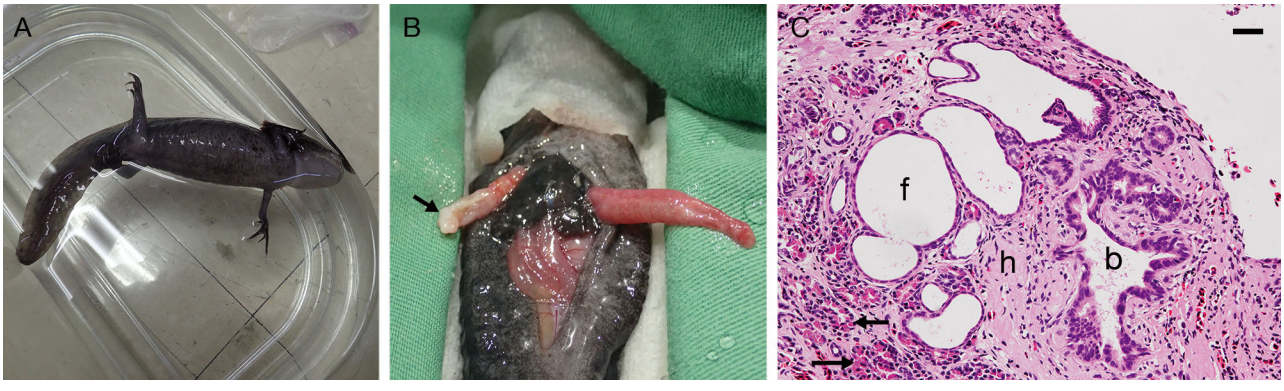


Fig. 2. *Ambystoma mexixanum* exhibiting abnormal buoyancy. Case 2. (A) Abnormal buoyancy in the water; right side of the body towards the top. (B) Right lung is white (arrow); macroscopic perforation site on the caudal side of the right lung. (C) Histology of the resected lungs. Epithelial cells lining the dilated lumen contained flattened (f) or similar bronchial epithelial cells (b). Ducts consisting of cells with a large number of eosinophilic granules (arrows) were also seen. Hyalinization (h) was observed in the expanded collagen fibers. Scale bar = 100 µm

hyperplasia of connective tissue and bronchial-like lumen lined with ciliated epithelium. The remaining alveoli had collapsed, and the condition was diagnosed as a non-infectious bronchiolitis (organizing pneumonia; Fig. 2C).

### Case 3

Case 3 was a leucistic male which had reportedly started to float on the surface of the water 18 mo prior to being presented. When the axolotl was brought to the veterinary hospital the right side of its body slanted towards the surface of the water, and this had reportedly begun to occur 1 wk prior to this time (Fig. 3A). The animal still had an appetite, but vomited after eating. Physical examination indicated swelling of the trunk that exceeded the head breadth. X-ray examinations and CT showed free air in the left side of the body cavity, and an acoustic shadow was detected via ultrasonography. Blood test results are shown in Table 1 (Takami & Une 2017).

In a gastrointestinal series, the contrast agent was excreted 10 h after ingestion, and there was no leakage of the contrast agent within the body cavity. The cause of the free air in the body cavity was tentatively diagnosed as a leak from the lungs rather than leakage of gastrointestinal gas, so the following day we performed an exploratory laparotomy under anesthesia. When a paramedian incision was performed, the gas retained in the body cavity disappeared.

The right lung adhered to the liver, and multiple yellowish-white nodules were scattered throughout the entire right lung. The tail end of the left lung had similar nodules (Fig. 3B). Macroscopically, neither the left lung nor the right lung had perforation sites. After separating the right lung from the liver, the affected areas of both lungs were ligated and resected. No macroscopic abnormalities were detected in other organs. Both aerobic and anaerobic bacterial culture tests using samples from the resected lungs were negative. After surgery, the subject was able to maintain a normal position at the bottom of the tank. The condition recurred 28 d after surgery, and the symptoms were seen intermittently after that, but the animal had an appetite and its general condition was good.

Histopathological tests on the resected lungs showed that both lungs had the same histological profile, and macroscopically visible nodules were formed from aggregations of inflammatory cells including histiocytes and lymphocytes in some areas. Within the interstitial connective tissue, which exhibited severe hyperplasia, there was formation of large and small lumens lined with columnar epithelial cells. There was also a scattering of severely expanded capillaries and macrophages with brown pigment, so the condition was diagnosed as obsolete multifocal granulomatous pneumonia (Fig. 3C). No pathogens were detected in the inflammation foci, including acid-fast bacilli.

## DISCUSSION

Buoyancy disorders are seen in fish and turtles with respiratory diseases, as well as marine mammals (Schmitt et al. 2005). In sea turtles, buoyancy disorders can be caused by pneumocoelom derived from gas production caused by lung damage, infections in the body cavity, intestinal impaction, and

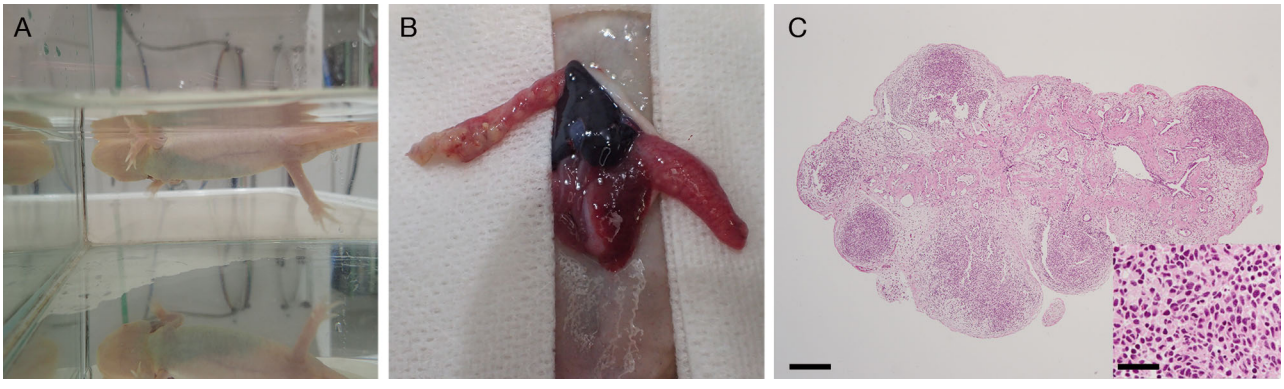


Fig. 3. *Ambystoma mexixanum* exhibiting abnormal buoyancy. Case 3. (A) Abnormal buoyancy in the water; right side of the body towards the top. (B) Right lung is adhered to the liver; multiple yellowish-white nodules scattered throughout the entire right lung. Tail end of the left lung had similar nodules. (C) Histology of resected lungs. Numerous granulomas detected at the periphery of the lung. Inset: higher magnification of one of the nodules. Scale bar = (main image) 1000 µm, (inset) 50 µm

nerve damage including spinal injury (Wyneken et al. 2006). There are reports that in ornamental fish buoyancy disorders can be caused by systemic granulomatous disease, abnormal fluid in the swim bladder, polycystic kidney disease, a ruptured swim bladder, and renal tumors (Gumpenberger et al. 2004, Wildgoose 2007). The results of the 3 cases in the current report suggest that one of the causes of buoyancy disorders in *Ambystoma mexicanum* is pneumocoelom caused by lung perforation derived from lung lesions. Lung samples from all 3 cases were examined microbiologically, and *Citrobacter* sp. was detected in Case 1, but the pathogen was not detected in histopathological tests. Therefore, the cause of the lung lesions could not be identified. In the future, using next-generation sequencing of lung samples, the cause might be detected.

In all 3 cases, blood biochemistry tests revealed high levels of ammonia. Ammonia toxicosis in amphibians is reported to cause dull skin coloration, mouth gaping and dyspnea, reddened skin, excess mucus, bright red gills, disorientation, seizures, failure to gain weight, and sudden death (Brent 2001, Wright 2006, Mitchell 2009). Zebra fish develop hyperammonemia when immersed in water containing a high concentration of ammonia (Feldman et al. 2014). However, the connection between amphibian ammonia toxicosis and hyperammonemia is unclear. It was not possible to clarify the relationship between buoyancy disorder and hyperammonemia in *A. mexicanum* within the scope of the current study, and blood biochemistry was not particularly helpful for diagnosis.

*A. mexicanum* perform gas exchange using paired external gills, their skin, and lungs. They have rudimentary lungs and at least 40–60% of their required oxygen may be obtained through these lungs (Whitford & Sherman 1968). Their lungs are elongated and semi-transparent, and they extend throughout the body cavity (Farkas & Monaghan 2015). Based on the authors' experience, it is possible to visualize a pair of lungs dorsoventrally via X-ray examination of healthy *A. mexicanum*, and acoustic shadows are almost never found within the body cavity via ultrasonography. When it is not possible to confirm a clear lung shadow via X-ray in *A. mexicanum* presenting with abnormal buoyancy, free air is found in the body cavity, and an acoustic shadow is detected via ultrasonography. These observations may be interpreted as signs of pneumocoelom caused by lung perforation. Thus, in such cases diagnostic imaging and exploratory laparotomy may be useful for investigating pathology.

Reports on the treatment of buoyancy disorders in other species include removal of the gas from the body cavity in sea turtles and management with fresh water and the administration of antibiotics (Wyneken et al. 2006). Reported treatments for fish include immersing the fish in sodium chloride salt at 2–5 g $l^{-1}$ as a permanent bath, increasing or decreasing the water temperature by a few degrees, fasting, feeding with finely crushed meal (Lewbart 2000), medical therapy such as the administration of antibiotics and the administration of acetazolamide, and surgery including the suctioning of gas from the swim bladder, and partial pneumocystectomy (Lewbart et al. 1995, Britt et al. 2002).

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