The Lungfish Spawning Event in 2009 at Logan's Inlet in Wivenhoe Reservoir

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Abstract

The spawning event involving lungfish in Wivenhoe Reservoir in 2009 has been described as a good news story, and evidence that lungfish are not endangered. This may not be the case. Although the fate of the young fish in the Reservoir is not known, none of the eggs collected in 2009 and reared in the laboratory survived. The spawning event was abnormal with eggs shed free into shallow water close to the shore where there were no water plants to shelter the eggs. Many of the eggs laid were not fertilised. Of the eggs that survived, some failed to develop and died before they could hatch. Some egg cases were fragile and released undeveloped embryos into the water. Under laboratory conditions, they survived, but in the wild, carnivorous aquatic insects would have quickly eaten these embryos. The young fish that were able to hatch at the correct time had no ciliated cells in the skin, and no way of keeping themselves clean in a foul environment. Sense organs in the skin were deficient, and many hatchlings had deformed mouths and intestines. The hatchlings were unable to feed and all died within eight weeks of hatching. The abnormalities and deaths were not the result of oxygen deficiency or elevated temperatures in the water. Eggs, embryos and hatchlings raised in the laboratory had sufficient oxygen, and temperatures were not elevated, and they all died. It is more likely that loss of the young fish is the result of a genetic problem in the parent fish, or deficiencies in the diet of the adults as they prepared for spawning.

Introduction

The Australian lungfish, *Neoceratodus forsteri*, is the sole survivor of a large group of related species that extended throughout eastern Australia in the Tertiary period (Kemp 2005). Most of these died out before the Pliocene, because of natural changes in the environment, and living lungfish are now confined to a few rivers and lakes in southeast Queensland. Natural populations of this fish are found in the Brisbane, Mary and Burnett Rivers (Lissone 2003, Kemp 1986, 2007) and translocated populations occur in the Pine River system, in the Coomera and Albert/Logan Rivers, and possibly in the Condamine River. Lungfish in the Condamine River may have suffered severely during the recent drought. Another translocated population, in Enoggera Reservoir, which spawned successfully for many years, has recently become extinct (Information from Brisbane Forest Park, confirmed by DPIF). The fish were very old, and no young had been recruited to the adult population because the water hyacinth on which the eggs were laid, and where the young were able to hide, was cleared from the reservoir in 1974 (Kemp 1995, 2007).

All populations of the living lungfish are under pressure from human interference with their environment and from natural events such as droughts and periodic flooding of the rivers and lakes in which they live. The living lungfish have survived the natural events, albeit with a significantly reduced distribution, but they may not survive human activities unless something is done to help them.

Creation of water impoundments and reservoirs in the rivers where the lungfish normally lives, such as the Burnett, Mary and Brisbane Rivers, has caused the loss of protected habitats for young juveniles, and reduced the amount of suitable food available for adult lungfish. In addition, adult lungfish are trapped in the water impoundments, which have fluctuating water levels. They are unable to move freely in the river system. These fish grow older and older, and, if recruitment is limited by loss of juvenile habitat, and other factors, the population is not renewed. Consequences of these changes are potentially devastating for the fish.

Lungfish return year after year to the same places to spawn if this is possible. They spawn annually in response to increasing photoperiod in spring (Kemp 1984). Although there is no parental care, a normal spawning event follows complex courtship rituals involving swimming rapidly together at the surface of the water, and

chasing each other through banks of water plants such as *Hydrilla verticillata*. When the fish spawn under normal circumstances, they are twined around each other with their vents close together, near water plants such as *H. verticillata* or *Vallisneria spiralis*, or to the submerged roots of trees like *Callistemon viminalis* growing on the river bank, or *Eichornia crassipes*, with its root masses suspended in the water (Kemp1984). As each egg is laid, the male sprays milt and fertilises it. Because the outer layer of the egg case is sticky for a short time, the egg will be attached to the closest plant or root mass, and is often quite deeply embedded.

Availability of water plants is not a trigger for spawning, although water plants increase the chances that eggs will be laid in a suitable environment if they are present. Lungfish will spawn even if the aquatic vegetation in their preferred locality is not there. Winter floods may remove the plants they normally use, like extensive beds of *Vallisneria spiralis* growing in the shallows, or damage the submerged roots of the *Callistemon* trees on the bank. If plants are missing for any reason, they will shed the eggs into the water column, and many will be washed into deep water and lost.

It follows that lungfish will spawn in a reservoir, even if conditions are not entirely suitable for eggs and young. They continued to spawn in Enoggera Reservoir after the water hyacinth was cleared, but in the absence of suitable submerged water plants for the eggs, nothing survived. The issue is not whether the fish are able to spawn in a water impoundment or reservoir, but whether successful recruitment of juveniles to the adult population can follow a spawning event in a reservoir where conditions are not natural.

Wivenhoe Reservoir was created from a part of the Brisbane River in 1984, trapping a large number of lungfish behind the wall of the reservoir. Prior to this time, lungfish had spawned regularly in the river and appropriate numbers of juveniles were recruited to the adult population. Lungfish may have continued to spawn in the reservoir, as they did downstream of the dam wall. The fate of any young lungfish produced in the reservoir is unknown, but conditions were less than ideal, especially during years of drought.

Previous work has suggested that refuges for lungfish hatchlings are essential to maintain the low level of recruitment needed for a species that can live for a long time (Bancroft 1913, 1918, 1933; Kemp 1995, 2007). Refuges made of submerged water plants or tree rootlets work well in an unaltered natural environment. However, few rivers in southeast Queensland are unaltered, and most are affected by the building of dams and reservoirs. These have fluctuating water levels and no permanent water plants in shallow water near the shore during spring when fish are trying to spawn. The spawning event of 2009 in Wivenhoe reservoir, and its outcome, suggests that lungfish in reservoirs face more problems than lack of shelter for the young, and are at risk from other factors such as poor parental technique, and possibly poor maternal nutrition, pollution or genetic defects in the parents.

The spawning environment in Wivenhoe Reservoir

Heavy rain in the winter of 2009 raised water levels in Wivenhoe Reservoir and submerged parts of the banks that had been exposed for many years. The water covered paddock grass and cow pats, and filamentous algae filled much of shallow water. Eggs were laid in still water of 30-80 cm depth, and temperatures at these depths were moderate, no higher than 22 ^{o}C at the time of collection. Temperatures of water in the shallows, around 5 cm deep, at the edge of the reservoir were higher during the day, up to 30 ^{o}C, and few eggs were found here.

Available habitats for small lungfish in the stagnant water of Wivenhoe Reservoir consisted of filamentous algae, rotting paddock grass and cow pooh. Suitable food was scarce, and predatory carnivorous insects were present in plague proportions. Temperatures at the time of spawning, between August and October, were moderate.

The spawning environment below Wivenhoe Reservoir

At Lowood on the Brisbane River, eggs were laid on the submerged rootlets of trees (*Callistemon viminalis*) growing on the river bank, and in full shade. Eggs were attached individually to the rootlets, often buried within the root mass, and at depths of 5-40 cm. Water flowed past the rootlets continuously, and temperatures varied

from 18-24 ^{o}C. Available habitats for young fish consisted of dense masses of rootlets, and these provided protection from the few predators that shared the environment. Suitable food for the young fish could also be found among the rootlets.

Spawning at this site ceased in 2002.

Collection and culture of lungfish eggs and embryos

Eggs were collected from Logan's Inlet by passing a small net through the water and searching the contents for eggs. These were transferred to plastic jars containing fresh cool water for transport to the laboratory. Stages of development, and condition of the eggs, were assessed on collection and on arrival in the laboratory. Numbers of dead and infertile eggs were also recorded on arrival, and on the day following collection. Material from spawning sites at Lowood that were collected under similar conditions, between the years 1986 and 2002, was used for comparison with the eggs from Logan's Inlet.

All eggs and embryos were reared in captivity by standard methods (Kemp 1981, 1999). Culture conditions for all of the fish were identical. The culture medium was prepared with rain water, there were weekly changes of one third of the medium in each dish, dishes were covered to prevent evaporation, and the eggs were not exposed to direct light. Water in the dishes was shallow, about 2 cm deep for eggs and embryos and 3-4 cm deep for older fish. Temperatures under which the eggs were raised ranged from 15-20 ^{o}C in late August, 16-24 ^{o}C in September, 18-27 ^{o}C in October and 20-28 ^{o}C in early November.

When the hatchlings reached stages where they could be expected to feed, they were provided with clean food, black worms or tubifex. Specimens from Logan's Inlet survived for about eight weeks after hatching, and died at around stages 47-48 (Kemp 1982). Material from the Brisbane River below Wivenhoe survived to sub adult stages, if not used for developmental research (Kemp 1999).

A series of eggs, embryos and hatchlings from each locality were fixed in 10% neutral buffered formalin and kept in formalin under refrigeration until they were required for analysis. Some of this material was set aside to serve as a reference collection, and a representative series of eggs, embryos and hatchlings were prepared for scanning electron microscopy (Kemp 1999).

Viability of material from Lowood

The percentage of eggs from Lowood that were dead on collection or died within one day of collection was 16%. Eggs collected in any one week during the spawning season were usually only a few days old, from stages 1-10 (Kemp 1982), with a smaller number of blastulae, up to 5 days old, and a few neurulae, up to one week old. There were always a small proportion of eggs that had reached stages of head development or were preparing for hatching, and even a few that were close to hatching. Very few empty egg cases were found at Lowood, and no newly hatched fish. However, these figures of older eggs were not a valid estimate of the number of eggs that reached hatching in the natural environment because collections of eggs were made every week in the same areas during the spawning season.

Viability of Logan's inlet material

The percentage of eggs from Logan's Inlet that were dead on collection or died within one day of collection was 45%. Most of the eggs collected from Logan's Inlet were young, and laid within the last day. There were also a number of neurulae, up to one week old, and a handful of eggs that had reached stages of head development, up to two weeks old. Hardly any were preparing for hatching, up to three weeks old, and none were at a stage when hatching usually occurs. Large numbers of empty egg cases were found, and no newly hatched fish. The egg cases were thin and fragile, and all had several large holes.

During the detailed study of part of the spawning site near Logan's Inlet, carried out by staff of the University of Queensland with the assistance of a field biologist employed by SEQ water, one hatchling, about stage 47, was discovered among the

muck and detritus on the substrate. This hatchling was taken back to the laboratory for rearing with the other young fish.

Eggs collected for the laboratory study came from areas remote from the study site. However, the distribution of eggs at particular stages of development was the same in the study site as it was in the site from which eggs were taken, with the exception of the single hatchling.

Progress of development of Lowood material

Eggs from Lowood took about four weeks to hatch, between stages 43 and 45. After hatching they passed through the normal early stages of inactivity, up to stage 47, and began to feed on black worms at around stage 48, becoming more active at stage 50 (Kemp 1982). During these stages of development, he range of sense organs in the skin and oral cavity increased from a few electroreceptors and mechanoreceptors in the snout, working in conjunction with the eyes and the olfactory organ inside the upper lip, to a complete sensory line system with many mechanoreceptors on the head and along the trunk, and many single electroreceptors scattered over the head, paired fins and trunk, as well as the lines of electroreceptors on the head. Eyes declined in importance as the young fish grew larger, and the olfactory organs and gustatory cells in the oral cavity became more significant. Cilia in the outer epidermis declined in numbers and activity as the fish became more active, and disappeared by stage 50. Losses of embryos and fish in the laboratory were few.

SEM of Lowood material

Egg membranes

At oviposition, the egg case is lined by a smooth double membrane with several deep folds but no holes or cracks (Fig. 1A). This is surrounded by a thick layer of albumen, also double. The outer layer, about one millimetre thick, is sticky when the egg is first laid, allowing the egg to be attached to nearby water plants or rootlets. This membrane collects dirt and debris rapidly and is soon no longer adhesive. The inner layer of albumen is 2 mm thick, and allows penetration by certain materials, such as

filamentous algae. The inner membranes are impermeable to algae or fungal hyphae. The egg is able to rotate within the membranes, at least until the segmentation cavity of the egg enlarges prior to the onset of gastrulation.

As the embryo grows, develops and begins to move independently within the egg capsule, the inner cavity expands. Cracks appear in the inner membranes, and these eventually break and fall to the bottom of the egg case (Kemp 1994). At the same time the albumen membranes are digested from within by an enzyme secreted by the embryo, and vigorous movements of the young fish enlarge the cavity further. Eventually holes appear in the albumen membranes and the hatchling is able to escape into the mass of plants or rootlets where it was laid.

Embryos and hatchlings

Differentiation of skin cells begins in embryos before hatching, and there is a thick plasmalemma on the external surface of all of the cells. Position and shapes of underlying structures, such as eyes, myotomes and the pronephros, can be discerned but they are not distinct. Many skin cells carry cilia (Figs. 2A, 3A). As the embryo develops further, ciliated cells increase, and the tail and intestines grow longer (Fig. 3A).

When the embryo is ready to hatch, several types of cells are visible on the skin surface (Fig. 4A). These are polygonal and similar in size and shape, between 20 and 25 microns across. Some of the cells are sensory, some have cilia and most are protective. The lateral line of the trunk, which appeared at stage 40, includes a number of mechanoreceptors, and these are present on the head as well, in the supra and infra orbital lines. Mechanoreceptors contain long stereocilia, and electroreceptors are single openings without stereocilia. Electroreceptors are scattered all over the skin of the snout, and above and below the lateral line of the trunk. The trunk lateral line reaches the tail tip by stage 43, and the sensory lines of the head are all present by stage 47 (Kemp 1999).

Cilia begin to develop at stage 30, and reach full size and activity at stage 40, declining after stage 48 when the hatchling becomes active (Kemp 1996). In these

hatchlings, ciliated cells are numerous and the cilia long and highly motile. Cilia on the head sweep particles over the head and away from the hatchling. Cilia on the trunk lie across the long axis of the cells, and are oriented oblique to the long axis of the hatchling so that the ciliary beat removes particles of dirt from the upper and lower surfaces of the small fish.

Scanning electron micrographs of embryos for this spawning site show little of the underlying structures unless they happen to be close to the skin surface, such as the tubules of the pronephros, or the eyes. Trunk muscles are present but not particularly obvious (Fig. 3A). Ventrally, there is a mass of endodermal cells that will form the intestine, and above this are traces of the underlying trunk musculature. The rectum, at right angles to the intestinal mass, opens into the vent. Anterior to the vent is a short fin fold, which disappears as the hatchling grows. The medial fins are of similar size below and above the lateral line, and form a wide triangular tail. The mouth is ventral and not quite terminal. In young fish (Fig. 3A), Prior to the development of the lips, it is open. Once the lips develop it is closed in fixed material (Fig. 5A). Pectoral fins develop from stage 44, and pelvic at stage 48.

Progress of development of Logan's inlet material

The course of development of Logan’s Inlet material differed significantly from that of Lowood material. A number of young eggs and embryos died before hatching for no obvious reason, and many developed fungal infections inside the egg case. Large numbers hatched at early stages, around 34-36. Hatchlings looked thin, and hardly moved at all, even when stimulated. Gradually yolk was used up, as is normal, but the hatchlings did not begin to feed when offered food, and they eventually died.

The young fish that hatched too early (around stages 34-36) had no developed sense organs and the muscular system was not capable of any co-ordinated movements. Eyes and olfactory organs had begun to form, but were not yet capable of receiving any sensory input. Electroreceptors and mechanoreceptors were completely absent, and all the hatchling could do was respond to repetitive noxious stimuli by flicking the head from side to side. At such early stages, the hatchling was incapable of searching for a suitable hiding place, or of choosing a habitat, even if one was

available. The remaining eggs from Logan's Inlet had all hatched by stage 40, still too young to be viable in the wild, and still without developed sensory organs. At this stage the hatchling should be able to move away when a harmful stimulus is applied, but the Logan's Inlet fish hardly moved at all, even when strongly irritated. Unlike hatchlings from the Brisbane River, they did not create a clear space around themselves when lying in a dish, indicating a lack of ciliary activity.

SEM of Logan's Inlet materials

Egg membranes

The inner layer of the egg membranes from Logan's Inlet has deep folds like eggs from Lowood. However, there are large gaps in the inner membranes, which expose the outer albumen (Fig. 1B). This allows enzymes from the skin of the hatchling (Hagenmeier 1974, Iuchi and Yamagami 1976) to attack the outer membranes too soon, producing large holes in the egg case and allowing the embryo to fall out of the egg before it is ready. The holes in the inner membranes, which should prevent the entry of pathogens, also made it possible for fungal spores (*Saprolegnia* sp.) to enter the egg case and infect the embryos. This explains some of the early prehatching losses.

Embryos and Hatchlings

All Logan's Inlet material examined so far had abnormal skin as well as other anomalies in structure and development. Skins of young embryos have a thin plasmalemma with a smooth surface and no apparent differentiation of skin cells (Fig. 2B, 3B). Cilia are not present in early stages when they should be developing. Some embryos have fungal hyphae emerging from the skin. In many embryos, brain and sense organs are abnormal in shape (Fig. 2B) and the intestine shows marked anomalies (Fig. 3B). The developing trunk musculature shows clearly through the thin skin.

In older hatchlings, skin cells show additional abnormalities. They may be arranged in whorls (Fig. 5C), and the cells are not normal in shape (Fig. 4B). There is little

differentiation into sensory cells, protective cells or ciliated cells, and the cell membranes are smooth. Cell sizes vary from less than 5 microns, well below normal, to around 30 microns and the shapes vary enormously (Fig. 4B). Most hatchlings have no ciliated cells on the skin surface. Cilia are present in some hatchlings, but are less numerous than they are in normal fish, and are carried on rounded cells that rise above the level of the epidermis, similar to developing cilia on much younger fish. The cilia, if present, are short and curled over, with masses of dirt obscuring their structure (Fig. 4B). Mechanoreceptors are scattered, and lack stereocilia, which should extend out of the mechanoreceptor. Electroreceptors are absent.

Head shape and size in relation to the length of the trunk is not correctly proportioned and the developing intestine is often abnormal in shape (Fig. 3B). There may be swellings in the intestinal mass or around the rectum. The vent may be entirely absent. The medial and paired fins are usually too small for the stage of the fish, and abnormal in shape. The operculum is often deformed, showing twisted and stunted gills that should be covered (Fig. 5B, C). Trunk musculature shows through the thin skin, as do sense organs that are normally obscured. The jaws are abnormal in most of the hatchlings examined, and the mouth should not gape (Fig. 5 B, C).

Discussion

The numbers of eggs that were dead on collection or died within a day of collection was much higher in material from Logan's Inlet, 45% compared with 16% of material from Lowood in the Brisbane River below Wivenhoe Reservoir. Many of these eggs would not have been fertilised, and some would have had one or another abnormality that caused early death (Kemp 1994). It is difficult to say if the egg was unfertilised because once it starts to decay, signs that suggest infertility disappear, and equally it is hard to say if the egg had cleavage abnormalities. However, this is unlikely to be an effect of temperature or of oxygen deprivation. High rates of infertility are likely to be a result of inappropriate spawning technique in the absence of dense banks of water plants or rootlets.

Some of the eggs and embryos from Logan's Inlet died because the egg membranes were deficient. In some cases, embryos hatched too soon because gaps developed in

the membranes before the embryo was old enough to hatch normally. In others, pathogenic fungi were able to enter the egg and infect the embryo. These problems result from inadequate structure in the egg case and are not related to environmental factors such as temperature or oxygen levels. In addition, many of the embryos were not developing normally prior to hatching.

The hatchlings from Logan's Inlet died because they did not develop normally, and the abnormalities were related to problems in cells of ectodermal or endodermal origin, that is in skin cells, cells lining the intestines, and organs based on ectodermal cells such as nervous tissues or parts of sense organs. Organs derived from mesodermal tissues, such as the pronephros or the myotomes of the trunk were less affected.

The specimens raised in captivity did not die because their environment was low in oxygen, or because the temperature of the water was too high. Material in the lab was not overheated and the water levels in the dishes allowed oxygen to diffuse into the water. Conditions in the reservoir were less favourable than those in the laboratory, but the eggs were in shallow water, and temperatures at the relevant time of year were not elevated. The structural changes in the eggs, embryos, hatchlings and egg membranes are too fundamental and too varied to have been caused by oxygen lack or elevated temperatures.

There are many possible explanations for the loss of hatchlings from Logan's Inlet. Lungfish have low genetic diversity, and some populations show a high level of abnormalities during development (Kemp 1994, 2003). The parents of the young fish may be too old to produce normal eggs and embryos. Pollution in the water of the reservoir is also possible. Diet of the adult lungfish may have been inadequate in the months before spawning.

The low genetic diversity of lungfish is well known, and the situation has existed for many years (Lissone 2003). They seem to have been able to survive this in many environments, including other rivers such as the Mary and Burnett, and in parts of the Brisbane River below the reservoir. A low genetic diversity of lungfish is universal, and not confined to the fish of Wivenhoe Reservoir.

It is true that abnormal development is present in some lungfish populations (Kemp 1994, 2003), but this does not usually cause the death of every single hatchling. Senescence in the parent population is not a probable explanation for loss of the young fish. Lungfish were trapped in the reservoir in 1984, but young lungfish could have come into the reservoir from spawning grounds in the upper Brisbane River where conditions are still characteristic of a riverine environment. Pollution from pesticides can also be ruled out. Although the banks of Wivenhoe Reservoir are used for raising beef cattle, modern farming techniques make use of insecticides that enter the animal directly, so pesticide run off into the water is unlikely to explain the loss of young fish. Nor do beef cattle require pastures enriched with fertilisers.

A possible explanation may be that the loss of the young fish may be a result of poor nutrition of the adults when they were preparing to spawn. Wivenhoe Reservoir has few small clams (*Corbiculina* sp.) and snails (*Plotiopsis balonnensis*). Adult lungfish rely on these for food. The intestines of the adult lungfish involved in the fish kill at the head of Wivenhoe reservoir in July 2009 were unusually empty, although the ovaries and testes were large. Poor food for parent lungfish could mean that any eggs laid could have had inadequate egg membranes and poor quality yolk to provide nourishment for the embryos and young hatchlings, and this could explain the suite of abnormalities found in the young fish in 2009.

Conditions in the reservoir in 2010 were similar to those in 2009, except that filamentous algae were absent and water levels were higher. Once again, there were no snails and small clams in the shallows. The lungfish did spawn in Logan's Inlet, in 2010, but only four eggs were found and none of these survived. Three were dead when collected, and the fourth died after a few days.

Suggestions for improvements in conditions for lungfish in Wivenhoe Reservoir

Reservoirs do not have steady levels of water, and it is not easy to maintain water plants to shelter small lungfish and supplies of snails and small clams in the shallows to provide food. In addition, water in a reservoir is stagnant, and overgrowth of

filamentous algae and of cyanobacteria is hard to prevent. Little can be done about the lack of flowing water in a reservoir, but it may be possible to build and maintain floating habitats in shallow water that contain, among artificial materials to provide sites for eggs and young fish, populations of *P. balonensis* and *Corbiculina* as food for adult fish. These floating habitats could be moved as water levels rise and fall, and may even allow water plants other than filamentous algae to become established among the artificial materials. I have tested artificial environments as spawning sites for lungfish, and they work, provided that they are close to sites that lungfish have used for spawning before. This solution need not be expensive, and once it is set up it is effectively self maintaining.

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Scanning electron micrographs of egg membranes, embryos and hatchlings of *Neoceratodus forsteri* from Lowood and from Logan's Inlet.

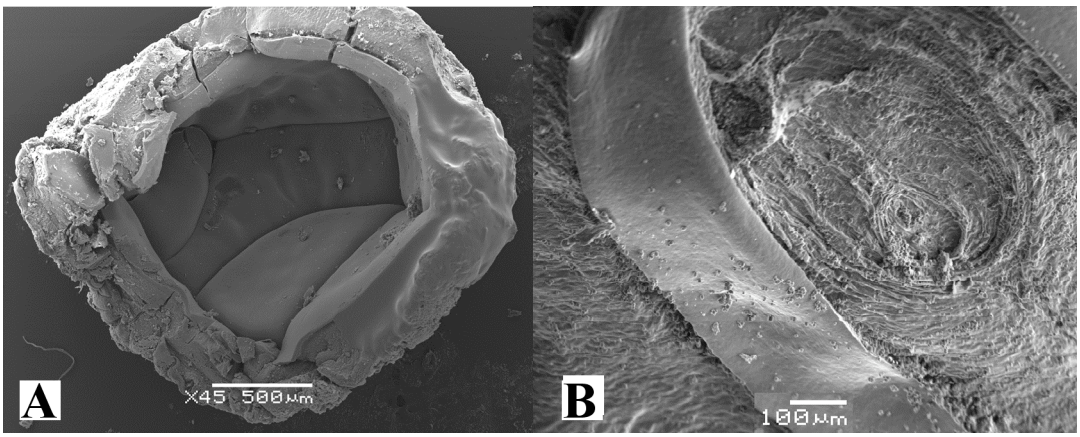

Figure 1. Lungfish egg cases. A. Normal, from Lowood, with complete inner membranes and thick albumen layers. B. Detail of deficient inner membranes in an egg case from Logan's Inlet, showing damage to underlying albumen.

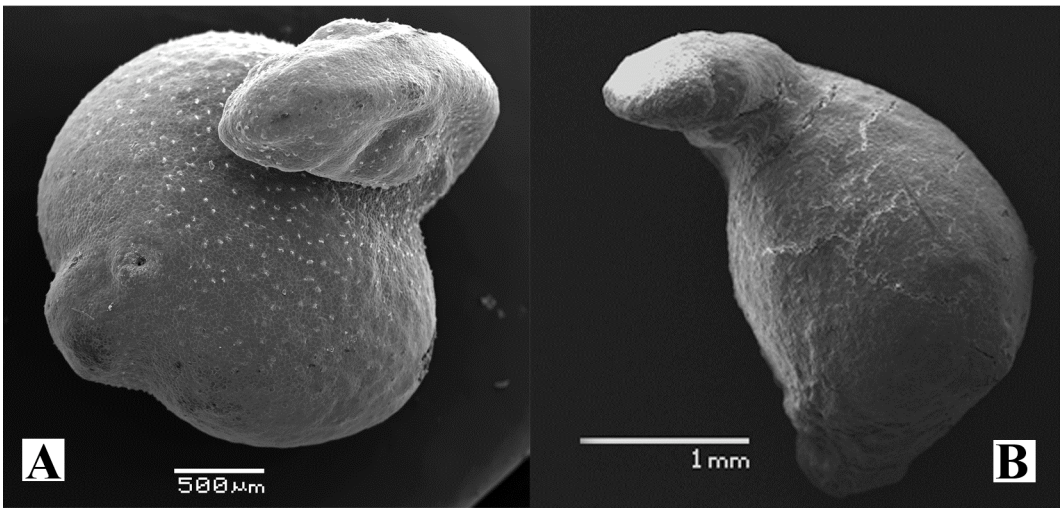

Figure 2. Developing lungfish embryos, about ten days old. A. Normal, from Lowood, showing head, tail and ciliated cells in the skin. B. Embryo from Logan's Inlet, with abnormal shape and no cilia. Cracks in the skin are a preparation artefact.

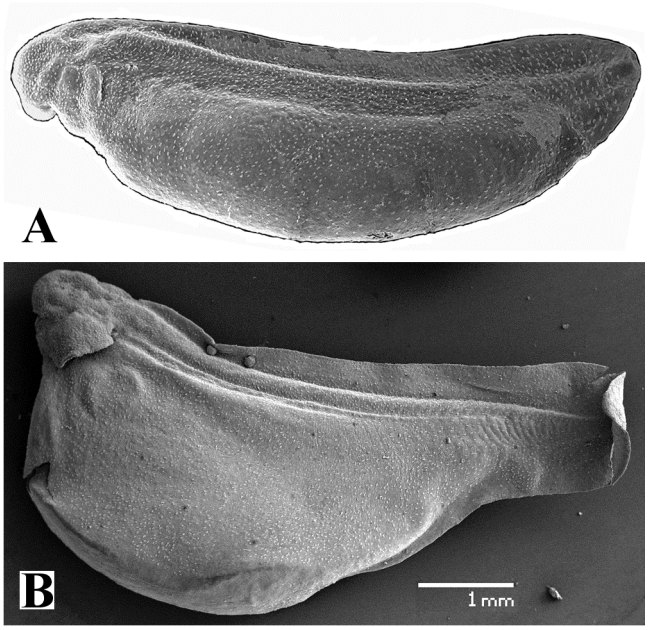

Figure 3. Lungfish embryos just before hatching, about three weeks old. A. Specimen from Lowood, with normal head, intestine and tail development. B. Specimen from Logan's Inlet, with deformed head and intestinal development.

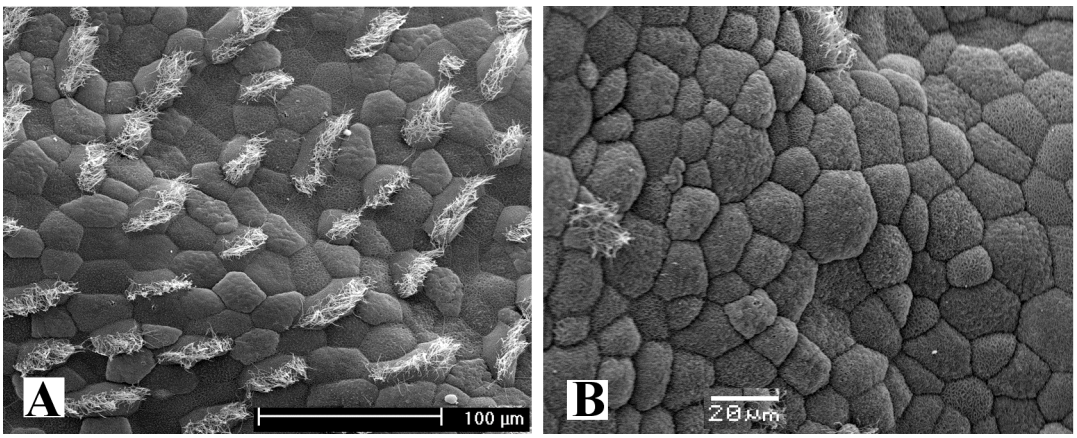

Figure 4. Skin cells from lungfish hatchlings A. From a hatchling collected as an egg at Lowood. B. From a hatchling collected as an egg from Logan's Inlet.

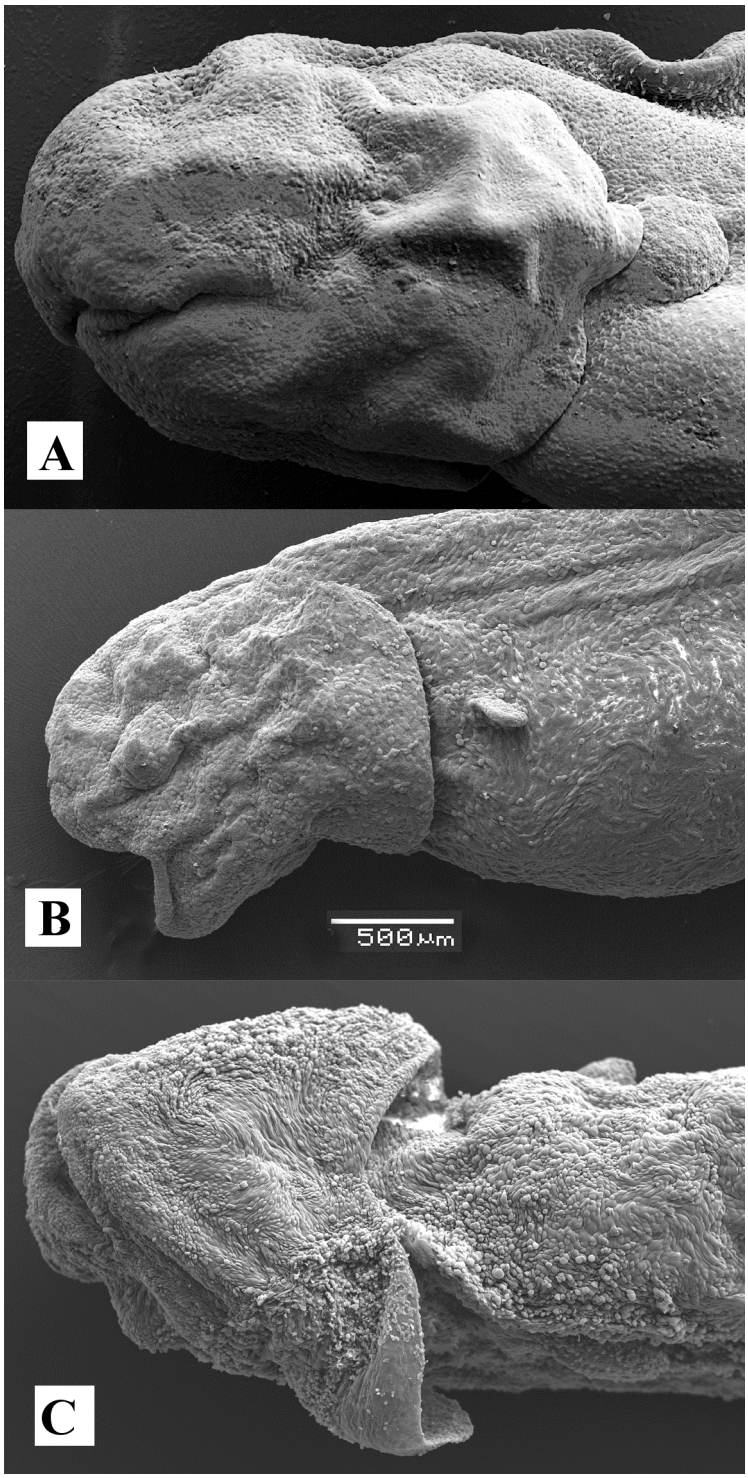

Figure 5. Small lungfish, about four weeks after hatching. A. Normal, from an egg collected at Lowood. B, C. Hatchlings from eggs collected at Logan's Inlet.