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Minimizing Fungal Invasion During the Artificial Incubation of Sea Turtle Eggs

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The artificial incubation of sea turtle eggs has become increasingly common for research purposes. It usually involves the collection of eggs, transport to a laboratory (potentially long distance), then incubation within a container and/or incubator on sand or an artificial substrate such as vermiculite. During incubation it may be necessary to inspect eggs to monitor development and mortality, and maintain moisture conditions.

At all stages during these procedures, eggs are exposed to infectants which have the potential to kill a proportion, or all, of the eggs. To minimize egg mortality, precautions can be taken to reduce egg exposure to microbiota.

Collection.—Collecting eggs directly from the ovipositor minimizes their exposure to fungal spores dispersed during the turtle's body-pitting and egg-chambering. Eggs may be caught by a gloved hand placed in the rear of the egg chamber (part of the chamber may need to be widened). Though some species are more tolerant than others, care must be taken not to disturb the turtle by use of excessive light, or by touching the ovipositor and hind flippers. If eggs cannot be collected during oviposition, they may be excavated after laying has concluded, but this method increases contact with soil microbiota. Some workers have attempted to place a collection bag in the egg chamber during oviposition. This often disturbs the turtle, resulting in nest abandonment, collapse of the egg chamber during bag insertion, and difficulty removing it when full. This method may be more successful with species that do not dig a deep body pit and/or on beaches with relatively moist sand, where the rear of the chamber may be excavated completely for easier insertion and retrieval.

Eggs should be placed directly into sterile bags; autoclave disposal bags (e.g., Sarstedt®) are recommended because of their strength. Placing the bag inside a bucket helps support the weight of the eggs and prevents weakening and tearing of the bag under stress. The neck of the bag should be twisted and folded over before securing. Prior to transport or incubation, eggs can be washed in sterile distilled water or a solution such as Aricide® (Hibberd 1996) to remove microbes from the egg exterior. However, washing removes the cloacal mucous and its potential anti-fungal properties (Phillott, unpubl.). After washing, the exterior of the egg should be patted dry using a clean disposable cloth to remove excess water and prevent ice-crystal formation and disruption of the shell structure during low-temperature transport.

Transport.—Eggs may be transported long distances by following the procedures of Harry and Limpus (1989). Eggs depressed to 7–10°C (within 2 h of oviposition) may be held for 48 h, allowing collection of multiple clutches over several nights and subsequent transport. It is recommended that eggs remain in the collection bags during this time to minimize exposure to microbes. The bags should be arranged so that they are stable, and air spaces filled with clean, expanded polystyrene pellets for support and insulation.

Handling.—To minimize movement induced mortality, egg orientation should be maintained (Limpus et al. 1979; Parmenter 1980). Single-use sterile gloves should be worn, and eggs handled in a room or area with minimal air flow or disturbance. If the area is to be used permanently for incubation purposes, a dual door system with an intermediate isolation area minimizes air disturbance during entry and exit. Workers must wait in the isolation area until the first door has completely closed before opening the second.

Incubation.—Prior to egg collection, incubators should be cleaned with a 5% sodium hypochlorite bleach solution, then rinsed with a 5% solution of disinfectant. Incubation containers (which will hold the eggs) may be sterilized by autoclaving at 121°C for 15 mins with the mouth covered in aluminium foil, or with a 5% bleach solution followed by a sterile water rinse.

If incubating on sand, it should be collected from areas relatively free of organic material. Sand can be sterilized by autoclaving in small lots. Thermal sentinels (e.g., Thermalog® S strips) should be used to ensure effective heat sterilization at the core. These checks can be ceased when the performance of particular autoclaves, sand types, etc., is quantified.

Labels on cardboard or other biodegradable material should not be placed in or on the substrate during incubation as they provide nutrient sources for mycobiota. Instead, the exterior of the container should be labeled. The required moisture conditions are maintained by adding sterile water either from clean spray bottles, or by use of a sterile water, sub-surface trickle irrigator (as described by Phillott and Parmenter 2001). If the plastic tubing from the latter is to be re-used, it should be first be cleaned with a commercial algaecide (used in cleaning swimming pools) then rinsed with sterile water.

When removing eggs (to weigh, measure, candle, etc.) wear sterile, single-use gloves and ensure the equipment is clean and that air flow around the area is minimal. If eggs are to be weighed, sand can be removed using a soft brush that will not damage the eggshell. Using a cloth to remove sand has the potential to drag sand across the egg surface, disrupting structural integrity of the eggshell. Eggs that fail to develop a white spot, that show signs of yellowing, or have fungal growth on the exterior should be re-

moved. Mass egg mortality often follows fungal contamination of a single moribund egg as hyphae spread to adjacent viable eggs (Phillott and Parmenter 2001). This potential spread can be minimized by ensuring eggs are not in contact with each other, though this is not always practical.

In the event of egg invasion, species of fungi can often suggest the source of the contamination. *Fusarium oxysporum*, *F. solani* and *Pseudallescheria boydii* have been regularly isolated as soil-borne pathogens on the exterior of failed eggs (Phillott et al. 2001). In contrast, *Aspergillus* spp. have been contracted by air-borne contamination (unpubl.). Identification of the fungi may lead to its point of source, allowing protocols to be modified so as to eliminate contamination.

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Captive Reproduction and Growth of the Broad-Striped Dwarf Siren (*Pseudobranchus s. striatus*)

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Dwarf sirens (*Pseudobranchus*) are aquatic salamanders endemic to the southeastern United States. There are two extant species, the southern dwarf siren (*P. axanthus*) and the northern dwarf siren (*P. striatus*). The northern dwarf siren consists of three subspecies (*P. s. striatus*, *P. s. lustricolus* and *P. s. sphenisus*) one of which, the broad-striped dwarf siren (*P. s. striatus*), is indigenous to South Carolina (Moler and Kezer 1993; Petranka 1998). The broad-striped dwarf siren inhabits cypress swamps and ponds in acid pine flatwoods, flooded ditches, marshes and other permanent and semi-permanent aquatic habitats from southeastern South Carolina to northern Florida (Conant and Collins 1991; Moler and Kezer 1993; Petranka 1998). Habitat loss and fragmentation as a result of development, agriculture, and silvaculture may be responsible for a decline of this salamander in South Carolina (S. Bennett, pers. comm.) Because of this, the South Carolina Department of Natural Resources has listed *P. striatus* as a state threatened species.

Most of the published life history data for *Pseudobranchus* species concerns *P. axanthus*. Although the life history of *P. striatus* may be similar to that of *P. axanthus*, for both species there is a general lack of data for growth rates, size and age at first reproduction, and many aspects of the juvenile and adult stages (Petranka 1998). According to Petranka (1998) there is "an almost complete lack of information on the natural history of *P. striatus*."

In 1994, Riverbanks Zoo and Garden attempted to establish a self-sustaining captive population of *P. striatus*. In July 1994, 0.0.6 *P. striatus* were collected in Hampton County, South Carolina and donated to RZG by the South Carolina Department of Natural Resources. Although the age of the specimens at the time of collection is unknown, it was latter determined that their small size corresponded with that of captive born young-of-the-year.

Husbandry.—The salamanders were initially housed in various glass and plastic aquaria ranging in size from 31 x 18 x 21 cm (LxWxH) to 50.8 x 25.4 x 34.2 cm. De-chlorinated tap water with a pH of ~ 7.0 was added to a depth of ~15.0 cm. Water temperatures were maintained between 23°C and 27°C. There was no mechanical, chemical, or biological filtration on any of the aquaria; however, 50% water changes were made weekly. Aquatic plants including duck weed (*Lemna* sp.) and bladderwort (*Urticularia* sp.) were added to all of the aquaria. A layer of substrate 2–4 cm deep consisting of small gravel, sphagnum moss, or leaf litter was added to some aquaria while others contained no substrate. Artificial lighting consisting of a 15-watt full spectrum fluorescent bulb and a 40-watt incandescent bulb was used on a single aquarium while the others had no supplementary lighting.

A variety of commercial, dry, flake fish food and live food was offered to the sirens ad lib. 3x/week. The preferred food item proved to be live blackworms (*Lumbriculus variegatus*) which were later fed exclusively. Live tubifex worms (*Tubifex* sp.) were not accepted as a food item.

The six wild-caught sirens readily acclimated to a captive environment and grew rapidly (Fig. 1). As far as could be determined, no one particular aquarium set-up proved to be more advantageous than another in terms of survival or growth rate.

Reproduction.—In May 1995, all individuals were assumed to be near adult size and an attempt was made to stimulate reproduction. *Pseudobranchus* are not considered sexually dimorphic. However, Petranka (1998) reports that "female *P. striatus* are on average slightly larger than males." Netting and Goin (1942) reported that in *P. axanthus*, the average total length (TL) of the five longest females they collected was 28% greater than that of the five longest males. The two longest animals at RZG (RZ 1260 and 1265), presumed to be females, and the two shortest (RZ 1261 and 1263), presumed to be males, were placed together in a 50.8 x 25.4 x 34.2 cm glass aquarium. Ambient water temperatures were