Anatomical Review and Standard Operating Procedure for the Atlantic Hagfish (*Myxine glutinosa*)

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Review of Hagfish Biology

Standard Operating Procedure

INTRODUCTION

Hagfishes (family *Myxinidae*) represent the most primitive craniates still in existence, and remain a valuable evolutionary resource which links the highly developed modern fishes to their ancient ancestors. Hagfishes lack many of the advanced characteristics present in more recent fishes, including jaws, paired fins (and associated internal cartilages), ribs, and true bones. The hagfishes share these primitive traits with only one other extant family of fishes, the lampreys (family *Petromyzontidae*). Together, they comprise the sole living representatives of the superclass *Agnatha* (*1*).

The Atlantic hagfish (*Myxine glutinosa*) is the most well studied hagfish of the 60 known species. It is a slender, elongate fish that resembles the lampreys and the true eels, although it can be easily distinguished from the first by a lack of an oral hood and the presence of anterior tentacles and from the second by the absence of pectoral fins and a bony jaw (Fig. 1). The length of the Atlantic hagfish ranges from 150 mm to 950 mm, making it the largest species of hagfish, although the average size is approximately 475 mm (*2*). The color of the animal varies based on

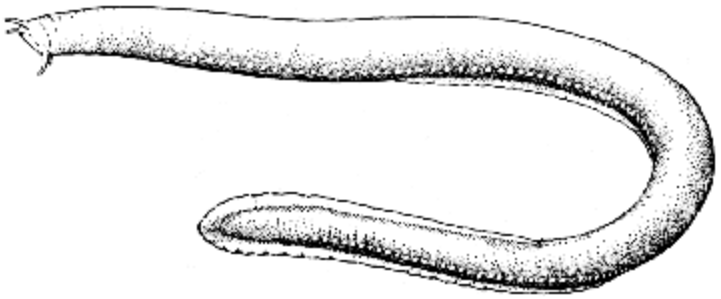

Figure 1: The Atlantic hagfish, *Myxine glutinosa* (from *2*).

the amount of oxygen in the blood, and the amount of blood present in the extensive network of dermal capillaries. While resting, the skin takes on a pinkish-grey hue that intensifies to bright pink with increased activity and finally deep purple with extreme exertion (*2*).

The range of the Atlantic hagfish extends from the eastern to the western North Atlantic, and as far north as Greenland to as far south as the Carolinas and the Mediterranean. The Atlantic hagfish is thought to be the most abundant hagfish, with observed densities approaching 500 000 individuals km^{-2}. However, population data for the Atlantic hagfish relative to other species might be skewed as much of the census data for this species has been collected from heavily fished regions of the North Atlantic. Nevertheless, such a high number signifies that the Atlantic hagfish must play a large role in the North Atlantic benthic ecosystem (*1*).

Since the hagfish has little nutritive appeal, except when used sparingly in Japanese sushi dishes, it has traditionally been considered a nuisance by most fishermen (*1*). More recently, however, demand by companies in South Korea for hagfish skins to be used in the manufacture of expensive leather goods has opened a lucrative market for American and Canadian fishermen, netting approximately $8.6 million between 1992 and 2002 (NMFS). In 2000 alone, 6.8 million pounds of hagfish were harvested, a number that represents a potentially serious impact on a species whose reproductive cycle and ecological importance remains to be fully defined. (*2*).

This review will briefly summarize the important anatomical and morphological characteristics of the Atlantic hagfish to date. These readings should supplement the material discussed in the included protocols. Further detail on most topics can be found by consulting the references.

PREBRANCHIAL REGION

The prebranchial region extends from the tip of the snout to the first gill pouch (*2*). The anterior-most structures of the hagfish are the pairs of nasal tentacles, followed by the oral and labial tentacles ventrally, which serve as both chemical and mechanical sensory receptors (*1*).

The hagfish is guided primarily by its sense of smell, and, as such, the subcutaneous eyes are small and degenerate (*2*). A single nasohypophasial aperture is located between the two nasal tentacles. Water is taken in through this opening and passes through the nasopharyngeal tract into the velar chamber, where the velum unfurls ventrally and laterally to deflect water into the pharynx. This process creates a vacuum into which more water is drawn, assisted by peristaltic contractions of the muscular gill pouches and their associated ducts. Water enters the gill pouches through the afferent duct after being flushed through the pharynx (*2*,*3*). While the hagfish is swimming, the nostril flares to allow for the unrestricted flow of water over the gills, and the tentacles are directed forward to probe the water (*2*).

The tri-partite brain of the hagfish is encased in a fibrous sheath, which is surrounded by the cartilaginous braincase. It is supported dorsally by a cartilaginous extension of the notochord (*1*). The forebrain is marked anteriorly by twin olfactory bulbs, which convey sensory inputs from the receptors at the front of the head to sensory targets in the telencephalon. The diencephalon, which contains the thalamus, epithalamus and hypothalamus, comprises the remainder of the forebrain (hagfishes lack pineal and parapineal glands). The forebrain tapers caudally into the mesenchephalon, which makes up the majority of the midbrain. The distinctly forked medulla composes the hindbrain, which diverges into two prominent horns where the sensory and motor trigeminal nerves intersect. The two lateral line nerves, the acoustic nerve, the combined glossopharyngeal and vagal nerves, and the reduced facial nerve are also connected to the medulla (*4*). Hagfishes possess both a spinal cord and a reduced notochord. The spinal cord is situated atop the notochord, and both emerge from underneath the medulla, flatten and continue dorsally along most of the body (*1*).

The hagfish circulatory system is comprised of an extensive network of sinuses which are connected on one side by arteries and on the other by veins (Fig. 2) (*1*). Hagfish have the greatest blood volume to body volume ratio of all the vertebrates, and > 30% of this blood resides in the sinus system. This type of partially open circulatory system also endows the hagfish with the lowest arterial blood pressure of all the vertebrates (*5*). As a result, circulation is aided by a number of accessory hearts, which independently supplement the pumping activity of the systemic heart.

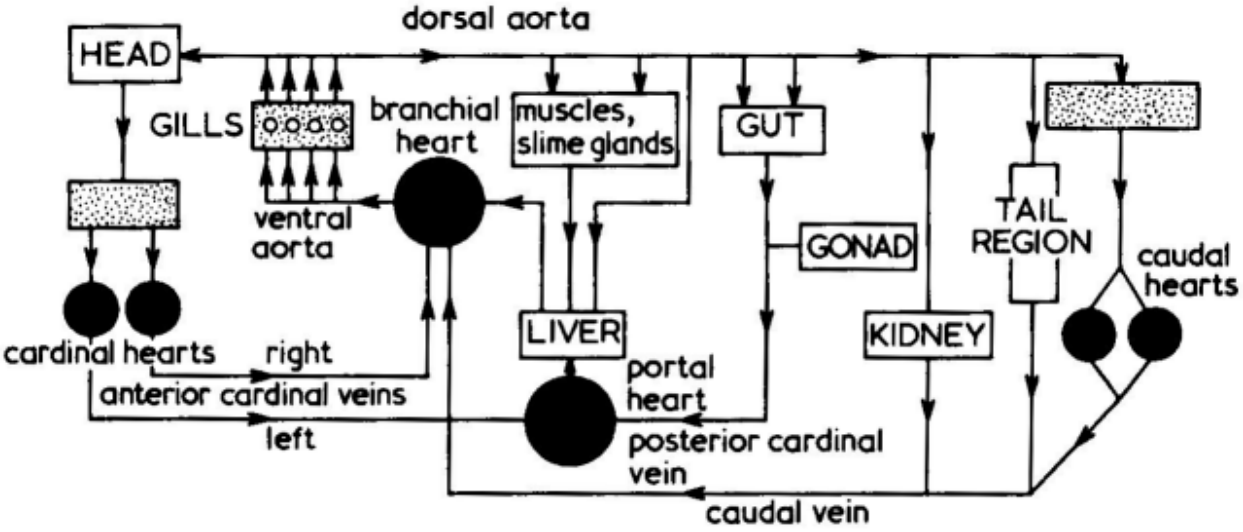

Figure 2: A schematic overview of the hagfish sinus system and its relation to the major pumping tissues (from *2*).

The cardinal hearts are the first of these accessory hearts, and are extensions of the anterior cardinal veins located between the velum and the brain. Because the cardinal hearts are driven by extrinsic muscle movements of the velum, they are not considered true hearts and can be better thought of as 'propulsors' (*1*). The cardinal hearts drive blood from the subcutaneous sinus (SCS), a large expandable cavity that contains the greatest amount of free blood present in the animal, into the anterior cardinal and inferior jugular veins. These channels return the blood to the portal and branchial hearts, where it is redistributed throughout the body (*1*, *6*).

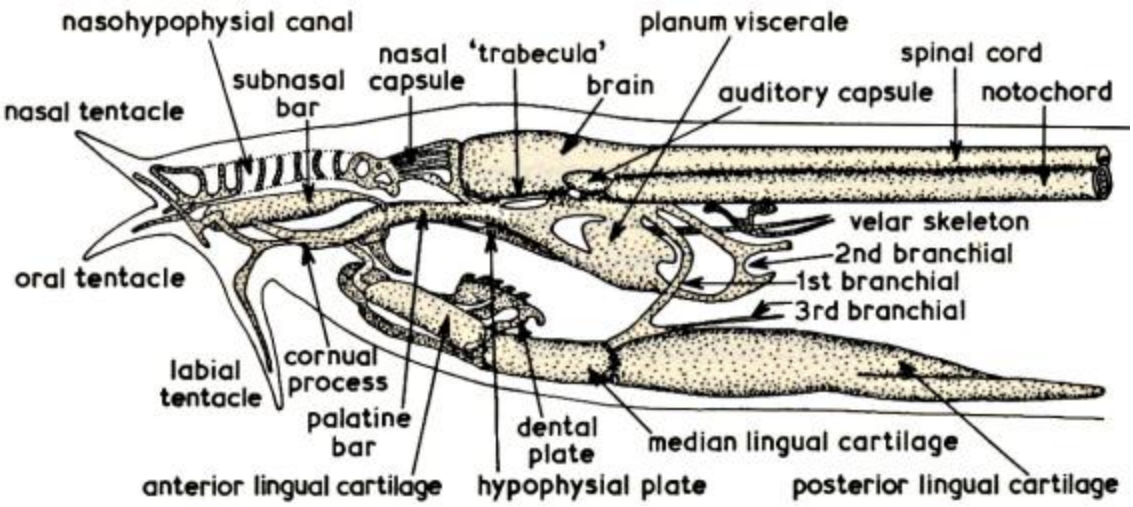

Figure 3: The head cartilages of the hagfish (from *1*).

The cartilages of the hagfish skull are fused to form continuous structures (Fig. 3). The dorsal longitudinal palatine bar extends anteriorly from the front of the braincase, ultimately thinning to become the cornual process that supports the cartilage of the tentacles. Directly above the cornual process is the large subnasal bar, which rests underneath the rings of cartilage which make up the nasohypophysial duct (*1*). The ear of the hagfish consists of two cartilaginous capsules found on either side of the medulla at the posterior end of the palatine bar. A single semicircular canal makes up the inner ear, rather than the two or more semicircular canals found in the higher vertebrates (*7*, *8*). The palatine commissure, carrying the palatine tooth, also connects to the end of the palatine bar (*1*). Three components comprise the tongue skeleton: the anterior, middle and posterior lingual segments. The anterior lingual cartilage consists of a single central bar and two lateral bars. This central bar bifurcates laterally and is continuous with the middle lingual segment. The first and third branchial arches are attached to the anterior of this middle segment. The second branchial arch is fused to the first and attached to the posterior of the velar plate, which supports the walls of the buccal cavity in front of the

velar folds. Finally, the posterior lingual segment is a long, flexible rod of hard cartilage that emerges from the posterior of the middle lingual cartilage and tapers to a point caudally (*9*).

Hagfishes lack true jaws. The funnel-shaped mouth is located ventrally, underneath the rostrum, and consists of an evertible dental plate with two rows of horny teeth on either side and the palatine tooth, a single curving tooth in the roof of the mouth. The diet of the hagfish consists primarily of benthic invertebrates. The hagfish consumes this type of soft or small prey by protracting the dental plate around the organism. The plate is then retracted, and the prey is guided into the pharynx through contact with the palatine tooth located on the interior surface of the palate (*1)*. An elongate organism can be ingested through this rapid grasp-retract-release-extend cycle (*2*). Once ingested, oral mucus, combined with repeated head depression and elevation, forces the food into the intestine where it is digested (*10*). Hagfishes also feed on large carrion that falls to the seafloor. The hagfish consumes this type of rough tissue by swimming vigorously headfirst into the carcass, and protracting and retracting the dental plate. When it has gained purchase, the hagfish ties itself into a knot and slides the knot along the length of its body. The movement of the knot over the head creates the force necessary to rip a piece of tissue away (*2*). Knotting also plays a large role in helping the hagfish to clear the body of accumulated mucus, slime and sediment. Without this behavior, the mucus would build up to such a degree that the hagfish would eventually suffocate itself. Knotting, mucus production, and general locomotion make it almost impossible to handle the hagfish, and therefore are believed to contribute to defense against predators and to deter competitors (*2*).

The slime glands of the hagfish begin in the anterior pharyngeal region and extend along the ventral midline almost to the tip of the tail. Each of the glands opens on the surface of the skin through individual ducts (*2*). These ducts exude holocrine secretions following the rupture

of the gland mucus or gland thread cell membrane, which is stimulated when the hagfish becomes irritated or excited (*11*). Hagfish mucus is composed of 99.996% seawater, 0.0015% mucin (a glycosylated protein), and 0.002% slime threads (protein fibers that greatly increase the strength and cohesion of mucus in the water) (*12*). The scaleless epidermis also contributes lesser merocrine secretions through normal exocytic discharge of mucus cells (*13*).

BRANCHIAL REGION

The branchial region extends from the first gill pouch to the posterior margin of the pharyngocutaneous duct (*2*). As the name suggests, the branchial region is comprised solely of respiratory structures that function in the exchange of gases. The primary component of the respiratory system is the lens-shaped gill pouches. Hagfishes are the only fishes with true gill pouches. The lampreys, which are closely associated with the hagfishes in many other aspects of their biology, have gills which more closely resemble those of the higher gnathostomes (*14*). The number of gill pouches is a useful tool in the classification of different hagfish species. The Atlantic hagfish is characterized by 6 gill pouches on each side of the pharynx, although a small percentage of abnormal specimens exhibit 7 or more pouches on each side (*2*).

Water enters the gill pouches through individual afferent ducts that originate from the pharynx. Water moves through the gill pouch along a central axis, parallel to which run a series of larger, first-order folds of epithelial tissue. The spaces between the first-order folds are filled with smaller, second- to sixth-order folds that gradually decrease in height as they move away from the central axis. These transverse folds are best developed in the center of the pouch, closest to the flow of water, and are absent from the regions closest to the medial and lateral walls of the pouch. The folds serve the same purpose as the gill filaments found in the lampreys and jawed fishes. Water exits the gill pouch through an efferent duct. The efferent ducts for the

6 gill pouches on either side of the pharynx converge and lead to two external, ventral gill pores. A ring of small cartilaginous plates surrounds the gill pores in order to keep them open and to assist the exit flow of water. The pharyngocutaneous duct, a direct connection between the posterior pharynx and the exterior, is located posterior to the last gill pouch and is fused with the common efferent duct on the left side (*14*). As a result, the left gill pore is noticeably larger than the right. The pharynogocutaenous duct is believed to aid the hagfish in clearing the pharynx of accumulated sediment or mucus (*2*).

Branchial circulation occurs through a series of afferent and efferent arteries surrounding the gill pouches (Fig. 4). Each gill pouch receives blood from an afferent gill artery stemming from the ventral aorta, which approaches the gill pouch from the lateral side and runs parallel to the external branchial duct. The afferent gill artery becomes an afferent ring artery, which surrounds the efferent branchial duct at the base of the gill pouch. Anastomotic afferent radial arteries arise from the afferent ring artery at regular intervals and form the base of the gill folds. These vessels supply sinusoidal cavities in the transverse gill folds with blood. Both the afferent radial arteries and the cavities, referred to collectively as the *corpora cavernosa*, are lined with epithelial cells that facilitate the exchange of materials. Efferent radial arteries draw replenished blood from the *corpora cavernosa* and converge at the efferent ring artery, which surrounds the internal branchial duct and is connected to the paired dorsal aorta by two efferent gill arteries. Such a system allows for the efficient countercurrent exchange of gases between the blood and the seawater (*14*).

It has been suggested that the skin might act as an important accessory gas exchange organ due to the large network of subepidermal capillaries. This idea, however, has been largely

discredited because the capillaries are already filled with blood that has a low capacity for additional oxygen. Furthermore, the hagfish spends most of its time burrowed 3-5 cm deep in

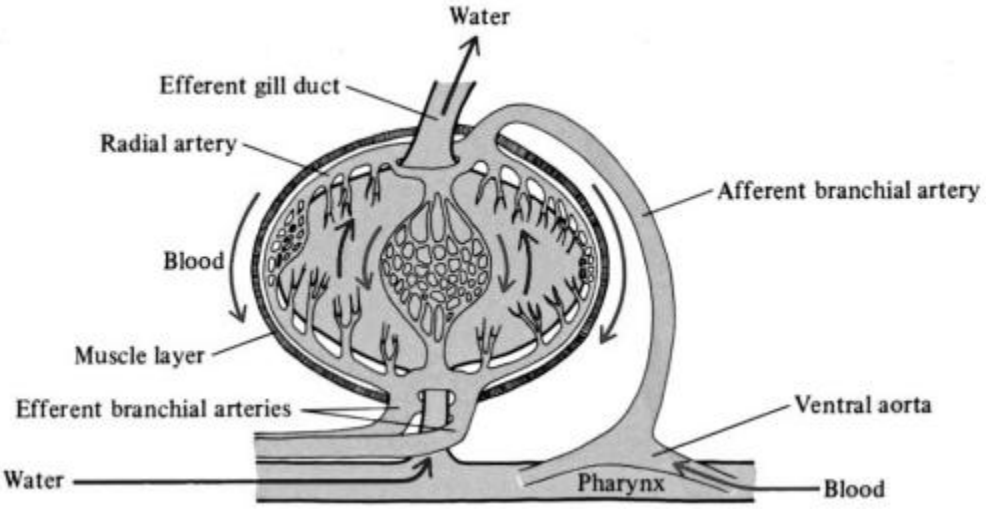

Figure 4: Branchial circulation through a gill pouch of the hagfish (from *15*).

the sediment. Oxygenated sediments extend only 5-6 mm below the surface, leaving most of the skin of the hagfish exposed to anoxic sediment that is unable to fully meet the oxygen needs of the animal. To maintain water flow through the gills, the hagfish lays parallel to the sediment surface and forcefully exhales through the nasohypophasial duct, creating a depression in the sediment where the head rests. A small mound of sediment kicked up by exhalation through the gill pores demarcates a hagfish burrow on the ocean floor (*14*).

TRUNK REGION

The trunk region extends from the posterior margin of the pharyngocutaneous duct to the posterior margin of the cloaca (*2*). The digestive system and its associated organs comprise the majority of the trunk region: ingested food moves through the pharynx and directly into the intestine. The intestine of the hagfish lacks cilia, and thus the passage of food through the gut is propelled by contractions of the body wall. The entire length of the intestine is uniform, and it is

presumed that all regions are involved equally in absorption. Additionally, portions of the mid-gut epithelium secrete digestive enzymes comparable to the exocrine functions of the pancreas in higher vertebrates, which remains undefined in hagfishes (*1*). The large, bi-lobed liver is located at the beginning of the intestine and also aids in digestion through the secretion of bile. Bile enters the gallbladder in between the cranial and caudal lobes of the liver through the hepatic ducts, where it is then delivered to the intestine through the bile duct. At the base of the bile duct is the islet organ, a small, white swelling of the intestinal tissue. The islet organ performs additional endocrine functions of the absent pancreas (*1*).

The hagfish lacks a defined spleen, but it has been shown that the liver might perform a similar function by sequestering red blood cells (*16*). It has also been proposed that the liver is the site of erythropoiesis (*17*). The liver plays additional roles in storage and in the detoxification of the blood. Blood pools in the liver after it has been distributed to the muscles by the dorsal aorta, or is pumped in by the portal heart after the blood has emptied into the posterior cardinal vein from the SCS (Fig. 2). The blood is driven through the liver by the portal heart into the branchial heart. Blood also enters the branchial heart through the caudal vein, which is supplied by the two caudal hearts in the tail region. From there, the branchial heart pumps blood through the ventral aorta and into the gill pouches (*1*). Both the portal and the branchial heart are composed of cardiac muscle, and are therefore considered true hearts. They do not function together, however, as their rates of beating are not synchronous. In fact, it has been found that the portal heart, which is unique to hagfishes, is not essential to survival (*5*).

Like the passage of food through the intestine, it is believed that the movement of blood from the SCS into the veins is primarily driven by contractions of the body wall (*5*). The segmented musculature of the hagfish runs the entire length of the body, and is composed of

205 >100 myotomes. A single myotome has one forward- and two backward-facing prongs that are directed obliquely backwards from the medial to the lateral surface such that the myotomes stack together. Each myotome is divided into a number of horizontal compartments that extend from the lateral to the medial surfaces. These compartments are further subdivided into: 2-4 layers of fast, white muscle fibers running horizontally under the surface of the skin to the medial border
210 of the myotome, and a single layer of slow, red muscle fibers over both the ventral and lateral surfaces (*1*). This intermingling of fibers is unique to the hagfish, as the red and white fibers are almost completely separated in the higher teleosts (*19*).

The hagfish swims at speeds of <2 knots using undulations of the entire body (*2*). Slow, sustained cruising utilizes the red muscle fibers, while short, quick bursts of speed are generated
215 by the white muscle fibers. The hagfish has a very low metabolic demand (2.39 kJ day^{-1}), and is therefore believed to be a sedentary, opportunistic scavenger with a narrow home range (*2*). Vigorous swimming has never been observed in the wild, and it is suspected that the hagfish spends most of its time under relatively hypoxic conditions, either buried in the sediment or feeding inside carrion (*19*).

220 Unlike the higher teleosts and some other species of hagfishes (*Epetretus* spp.), the Atlantic hagfish has no trace of a lateral line system. It does, however, have a well-developed chemosensory system composed of many individual Schreiner organs. A single Schreiner organ consists of a pear-shaped aggregate of cells, which can be densely clustered or loosely dispersed, with microvillar projections. The Schreiner organs are concentrated anteriorly, with the highest
225 densities found on the tentacles and surrounding the mouth and rostrum. They are present in a moderate, uniform density from the middle of the head rearward, but are reduced caudally, as well as dorsally, with the lowest densities found on the caudal fin. Schreiner organs are also

found within the nasopharyngeal duct and the pharynx. It is estimated that there may be >180 000 Schreiner organs found on the hagfish *E. stoutii*, although this number might be lower for *M. glutinosa* (*20*).

A hagfish reaches sexual maturity when it is >400 mm in length. Hagfishes are not simultaneous hermaphrodites, but they do have both male and female gonadal tissues. The ovary is usually found in the anterior two-thirds of the dorsal mesentery and the testis in the posterior one-third. An individual hagfish may have an immature testis and ovary, an immature testis and a mature ovary, or a mature testis and an immature ovary. A recent study of the hagfish population of the North Atlantic has shown that: approximately 59% of the population is female (as determined by the development of eggs and the reduction of testicular tissues), less than 6% of the population is male, and approximately 25% of the population is sterile (*2*).

A female hagfish will produce about 30 eggs per clutch. The maturing eggs develop a number of short filaments at the poles that end in hooks, which help to anchor the eggs to the walls of the coelom and to each other. It is thus postulated that the eggs are released in clumps. The time it takes to produce a single clutch is unknown. Since many female specimens exhibit eggs in all stages of oogenesis, it is currently believed that hagfish have no defined breeding season (*2*). However, recent evidence has shown that the seasonal production of gonadotropin-releasing hormone (GnRH), a major hypothalamic neurohormone involved in mediating reproductive activity in all vertebrates, may in fact seasonally guide the reproduction of the Atlantic hagfish (*21*). However, due to the high percentage of sterile individuals and the low number of mature eggs present in a female at any given time, the reproduction of hagfishes is likely to be very slow (*2*).

The method of fertilization is unknown. Self-fertilization is unlikely, as egg-producing females generally have reduced testicular tissue. However, whether fertilization is internal or external remains contested. Some believe that internal fertilization is not likely, as males do not have a copulatory organ and the cloacal epithelium of the females is devoid of any structures that might aid in the capture of sperm. In this case, both eggs and sperm would be released in close proximity and fertilization would be external, although the site of egg deposition remains a mystery. A small number of fertilized eggs have been recovered from soft bottom sediments in the eastern North Atlantic. It has therefore been proposed that eggs are laid within the burrow of the hagfish, and cutaneous exchange between the parent and the anoxic water of the burrow would provide sufficient oxygen for the developing embryos (*2*). Alternatively, others argue that the cloacal gland, a long, mucus-filled tube that is located dorsally to the rectum, may play a role in the capture of sperm despite the absence of any external structures. It has been shown that the cloacal gland of females that had recently mated contained many degenerate nuclei, a characteristic that has yet to be induced in other specimens. It was proposed that these pycnotic nuclei once represented sperm cells that had been taken up and incorporated into the mucus of the cloacal gland. As this mucus was released to coat the eggs and ease their release from the cloaca, the sperm would have also been deposited and fertilized the eggs while still inside the animal (*22*).

Hagfishes are osmoconformers, and they maintain an internal solute concentration that is isotonic to their environment. As a result, hagfishes experience neither a passive influx of water as in the freshwater fishes nor an efflux of water as in the saltwater fishes. Hagfish must therefore actively regulate their ion concentrations and flush out excess compounds using their kidney (*23*). The mesonephric kidney of the hagfish is composed of a pair of archinephric ducts

(AND) which lie on either side of the midline. Each duct is joined by a single glomerulus for each segment. However, in the more caudal regions, the glomeruli may be absent, and in the cranium the ducts end blindly and also lack glomeruli (*1*, *24*).

The flow of blood through the kidney remains to be fully characterized. A pair of arteries carries blood from the dorsal aorta laterally and dorsally to the body wall. Small renal arteries branch off of the segmental arteries and pass through the glomerulus. Blood enters the AND through the glomerulus, or through small arteries which arise from the segmental arteries lateral to the renal arteries. Blood flow in the glomerulus occurs in both superficial and deep vessels, which can be interconnected. Ion exchange occurs within the glomerulus, generating the urine that is expelled where the AND converge at the cloaca. Blood drains from the walls of the AND into small segmental veins, which converge at the caudal veins (*24*).

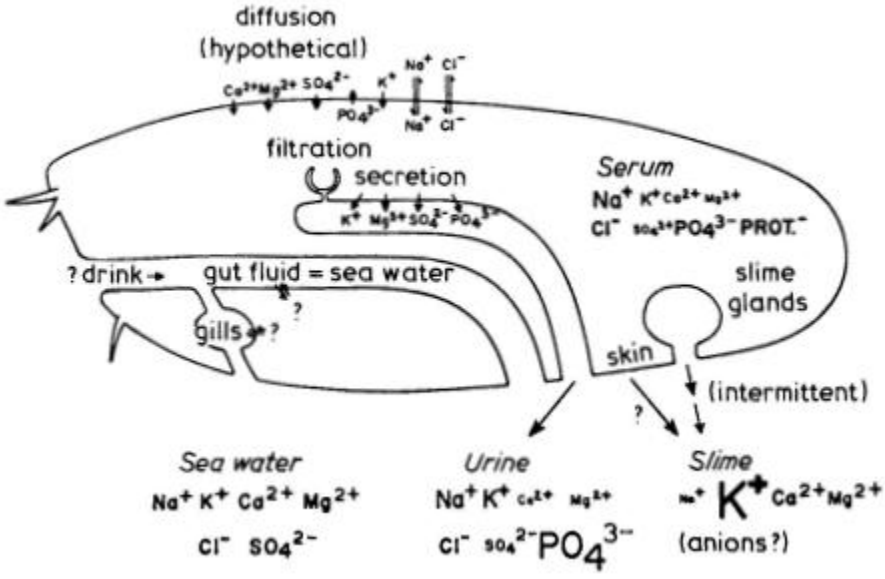

Figure 5: Proposed mechanisms of ionic regulation. The size of the text for the various ions indicates their concentration relative to the seawater. Relative permeability is indicated by the lengths of the arrows (from *1*).

There are significant differences between the ionic composition of hagfish blood and that of seawater (Fig. 5). As a result, hagfish urine is composed of those ions found in higher concentrations in seawater, such as potassium, magnesium, sulfate and phosphate. Similarly, the

kidneys retain other ions, such as sodium, that are found in lower concentrations in seawater. However, it remains unclear whether the kidneys have the ability to recover ions once they have entered the glomerular filtrate. Other sites of ion excretion may include calcium and magnesium in the bile, and calcium, magnesium and potassium in mucus secretions of the slime glands (*1*).

CAUDAL REGION

The caudal region extends from the posterior margin of the cloaca to the tip of the tail. The single median finfold of the hagfish, which begins immediately posterior to the gill pores, terminates in this region dorsal to the cloaca (*2*). This region is anatomically dominated by the bilateral caudal hearts. Blood enters the hearts through valved openings from the marginal vein of the fine membrane of the tail and from the SCS, and empties into the caudal vein which returns it to the branchial heart. The muscles of each heart contract alternately and are active intermittently. Pumping is initiated shortly after the hagfish stops swimming and high blood pressure has moved a large volume of blood into the SCS which needs to be returned to central circulation. There is evidence, however, that blood is moved through the hearts in the absence of muscular contractions simply from increased blood pressure. Thus, it is uncertain whether muscle activity is truly correlated with blood volume (*5*).

CONCLUSION

While much is known of the biology of the Atlantic hagfish (*Myxine glutinosa*), it is clear that further research is necessary to fully characterize this important ecological and evolutionary creature. It is hoped that this protocol may assist others in accomplishing this goal.

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Species: Atlantic hagfish (*Myxine glutinosa*)

Veterinary Approval:

Procedure Type: Major surgery
Procedure Name: FINQUEL® (MS-222) and potassium chloride (KCl) euthanasia preceding dissection of Atlantic hagfish (*Myxine glutinosa*)
Procedure Description: Terminal procedure followed by dissection.
Estimated Procedure Duration: Setup and calibration of materials – 20 min; anesthetization – 30 min; surgery – 30 min; data and recording keeping – 10 min. Total time – 1 hr 30 min.

MATERIALS

Tools	Chemicals/drugs/doses
3.8 L (1 US gallon) container with lid	500 mg L^{-1} FINQUEL® MS-222 (tricaine methylsulfonate), Argent Chemical Laboratories
Centigrade thermometer	
Laboratory scale	1000 mg L^{-1} sodium bicarbonate ($NaHCO_3$) Buffer
Autoclaved surgical instruments:	2.5 g L^{-1} potassium chloride (KCl)
Operating scissors (sharp/blunt; Miltex #4698)	
Iris scissors (Miltex #4059)	
Splinter forceps (Miltex #4516)	
#3 scalpel handle (Miltex #4054) #12 blade	
Mayo-Hegar needle holder (Miltex #4084)	
Metric ruler	
Latex gloves	

ANESTHESIA

Medications administered to an animal, including fishes, must be authorized by a licensed veterinarian. FINQUEL® has been approved by the FDA for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals. This procedure will administer a lethal dose of FINQUEL®. The AVMA has strict guidelines regarding ethical methods of

euthanization. It is recommended that you not stray from this SOP in order to uphold these standards.

PRE-ANESTHETIC EXAMINATION

Evaluate the health status of the fish to be anesthetized prior to preparing the solution. For more information regarding the health status of anesthetic candidates see Skarda et al. 1995; Stoskopf 1993.

STAGES OF ANESTHESIA

Determine the stage of anesthesia intended for use during this procedure (Table 1). For more information regarding anesthesia in fishes see Stoskopf 1993; Kikasa et al. 1986; Machin 2001.

Table 1: Stages and planes of anesthesia for different procedures.

STAGE	PLANE	CATEGORY	BEHAVIORAL RESPONSE OF FISH
0		**Normal**	Swimming actively, reactive to external stimuli, equilibrium normal, muscle tone normal.
I	1	Light sedation	Voluntary swimming continues, slight loss of reactivity to visual and tactile stimuli, respiratory rate normal, equilibrium normal, muscle tone normal.
I	2	Deep sedation	Voluntary swimming stopped, total loss of reactivity to visual and tactile stimuli, slight decrease in respiratory rate, equilibrium normal, muscle tone slightly decreased , still responds to positional changes.
II	1	Light narcosis	Excitement phase may precede increase in respiratory rate, loss of equilibrium, efforts to right itself, muscle tone decreased, still responds to positional changes weakly.
II	2	Deep narcosis	Ceases to respond to positional changes, decrease in respiratory rate to approximately normal, total loss of equilibrium, no efforts to right itself, muscle tone decreased, some reactivity to strong tactile and vibrational stimuli
III	1	Light anesthesia	Total loss of muscle tone, responds to deep pressure, further decrease in respiratory rate, suitable for minor surgical procedures.
III	2	Surgical Anesthesia	Total loss of reactivity, respiratory rate very low, heart rate slow.
IV		**Medullary collapse**	Total loss of gill movement followed in several minutes by cardiac arrest

DATA RECORDING

A record of each anesthesia procedure must be kept and available for inspection.

PERSONAL SAFETY

Before beginning this procedure, read this SOP including the attached manufacturer's instructions and the material safety data sheet for each compound. Use of these products may be hazardous to your health. Proper safety equipment should be used to prevent contact and

inhalation. Do not proceed with this SOP until you understand these directions and have taken the proper precautions. Be aware of the risks involved with handling live animals.

EUTHANASIA PROTOCOL

1.) Take 3.8 L container and fill with 3.5 L of saltwater at 10°C. Ice can be added in small increments until this temperature is achieved; additional salt can also be added to restore the salinity to about 35 ppt.

2.) Determine the region in which the target tissue is located, and proceed appropriately:

Target tissue within pre-branchial region (3-9)

3.) Weigh out 0.875 g of FINQUEL® MS-222 (tricaine methylsulfonate) and 3.500 g of sodium bicarbonate ($NaHCO_3$), and add to container. Mix thoroughly until powder is uniformly dissolved.

4.) Weigh out 7.500 g of potassium chloride and add to solution. Mix thoroughly until powder is uniformly dissolved.

5.) Carefully submerge the specimen in the container and secure the lid tightly.

6.) Observe the specimen until it shows signs of entering the stage of surgical anesthesia, indicated by a lack of response to external stimuli. This should occur after approximately 10 min. It is recommended that 20-30 min pass before the specimen is handled.

7.) Remove the specimen from container and confirm that it is properly euthanized. Residual muscular contractions as a result of the anesthesia protocol are common.

8.) Exsanguinate the specimen by using the operating scissors to completely remove a small portion of the tail. Exsanguination should relieve the intermittent twitching.

9.) Properly dispose of chemical waste.

Target tissue not within pre-branchial region (3-8)

3.) Weigh out 1.75 g of FINQUEL® MS-222 (tricaine methylsulfonate) and 3.500 g of sodium bicarbonate ($NaHCO_3$), and add to container. Mix thoroughly until powder is uniformly dissolved.

4.) Carefully submerge the specimen in the container and secure the lid tightly.

5.) Observe the specimen until it shows signs of entering the stage of surgical anesthesia, indicated by a lack of response to external stimuli. This should occur after approximately 10 min. It is recommended that 20-30 min pass before the specimen is handled.

6.) Remove the specimen from container and confirm that it is properly euthanized. Residual muscular contractions as a result of the anesthesia protocol are common.

7.) Using the operating scissors, quickly and completely decapitate the specimen just anterior to the gill openings. This should prevent residual muscle contractions that may occur as a result of the anesthesia protocol.

8.) Properly dispose of chemical waste.

SAMPLE DISSECTION PROTOCOL

1.) Obtained euthanized specimen:

Date: 04/18/08

Fish Species: Atlantic hagfish (*Myxine glutinosa*)

Number/History: 1, received from Mount Desert Island Biological Laboratories on 04/18/08

Sex: H

Size: 455 mm, tapers anteriorly

Color: Mottled light pink to deep purple

2.) Examined and noted external characteristics.

External

Skin: Scaleless

Fin: Single medial finfold begins directly posterior to gill pores, circles caudally and terminates dorsally at the level of the cloaca

Mucus pores: Approximately 130 small white pores running the length of the animal

Gill pores: Two ventrally, located approximately 125 mm from rostrum

Mouth: Located ventrally; four folds of tissue converge at central point; flanked by labial tentacles

Tentacles: Three pairs, moving dorsal to ventral: nasal, oral and labial

Nare: Conspicuous nasohypophasial aperture between two oral tentacles

Cloaca: 10 mm slit found approximately 365 mm from rostrum

3.) Made ventral incision from the left gill pore and cut posteriorly for the length of the animal.

4.) Made second ventral incision from the left gill pore and cut anteriorly to the base of the mouth. Folded back skin to reveal coelom and associated internal organs.

5.) Made third incision at base of cranium and removed skin anteriorly to nasohypophasial aperture to reveal dorsal head cartilages.

6.) Made fourth and fifth incisions along the side of the head and folded back the top layer of cartilage to reveal the brain, approximately 20 mm from rostrum, and other associated internal head cartilages.

Internal	**Brain:**	White mass located approximately 20 mm from rostrum
	Lingual cartilages:	Directly posterior to the mouth, overlays the pharynx; posterior lingual cartilage largest and most obvious
	Pharynx:	Thin white tube, visible by removal of lingual cartilages, gill pouches
	Gill pouches:	Series of lens-shaped, red sacs; 6 on either side of the pharynx
	Musculature:	Approximately 85 myotomes running the length of the trunk
	Branchial Heart:	Located dorsally to the liver, smaller
	Portal Heart:	Located ventrally to the liver, larger
	Branchial Hearts:	Bilateral, located on either side of the brain
	Caudal Hearts:	Bilateral, located in viscerum at posterior of tail
	Liver:	Large, conspicuous brown organ with obvious venation
	Gallbladder:	Within liver, conspicuous dark blue sac
	Intestine:	Long, flesh-colored tube running from the liver for the rest of the length of the animal
	Reproductive:	White, capsule-like eggs of varying sizes found alongside the intestine
	Cloacal gland:	Enlarged white organ of soft consistency found at posterior of coelom

7.) Properly disposed of specimen, and cleaned tools.

Notes: Specimen stressed due to transport. Large cloacal gland with soft consistency may indicate that the specimen may have mated or been preparing to mate.

FIGURES

Fig. 1: Specimen of Atlantic hagfish (*Myxine glutinosa*) before dissection procedure. Scale indicates 1 cm.

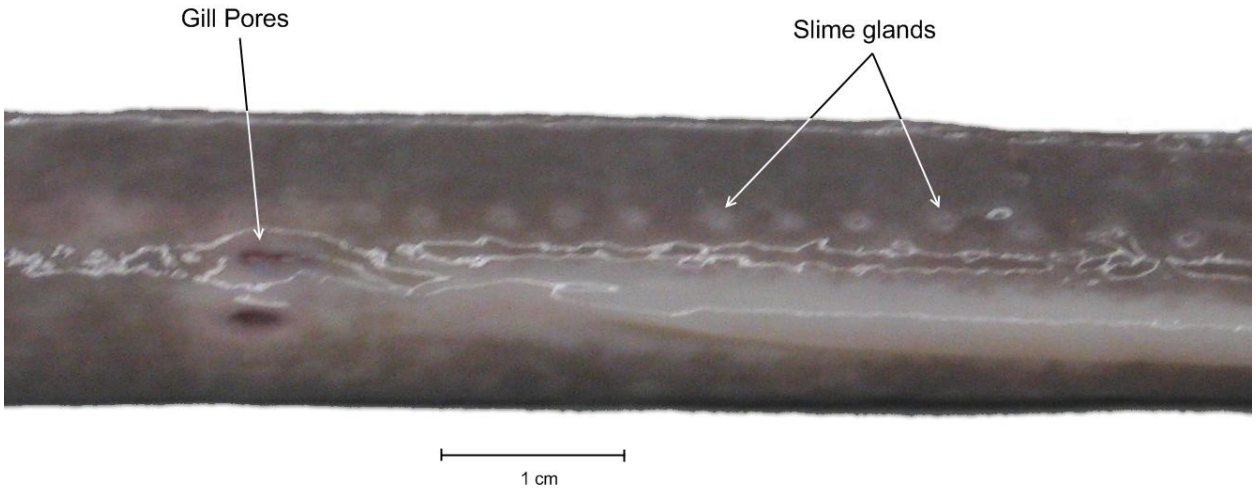

Fig 2: Ventral features of the mid-trunk. To the left are the two gill pores; the left (upper) gill pore is noticeably larger than the right. The pores of the slime glands are visible as a row of white dots. The single finfold can be seen as the white band emerging immediately posterior to the gill pores. Scale indicates 1 cm.

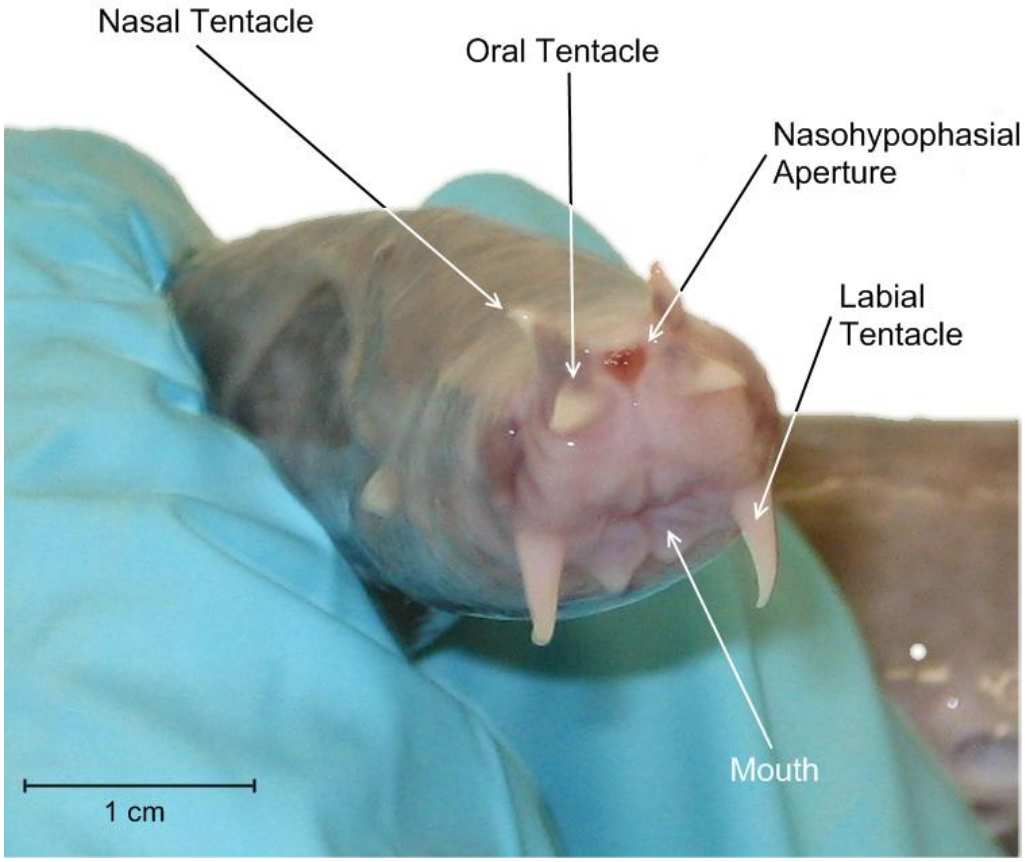

Fig 3: External features of the head. The three pairs of tentacles are indicated (moving dorsally to ventrally): nasal, oral and labial. The nasohypophasial aperture, which opens into the nasohypophasial duct, is observable between the two pairs of nasal and oral tentacles. The mouth is visible as a fold of tissues flanked by the labial tentacles. Scale indicates 1 cm.

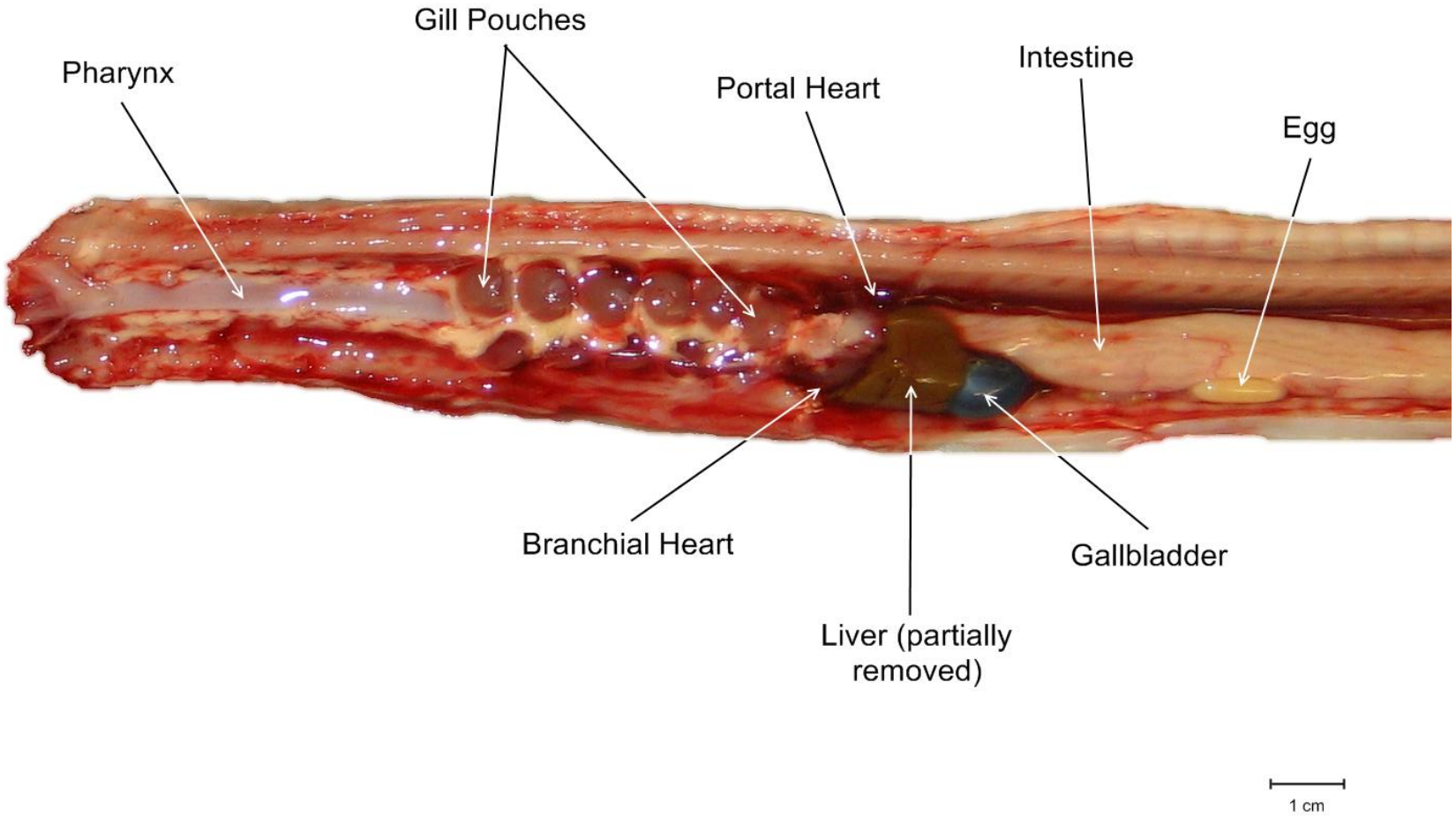

Fig. 4: The internal structure revealed after the first two incisions. The lingual cartilages have been removed to show the pharynx, which travels underneath the gill pouches and merges with the intestine at the point of the liver. The two true hearts (portal and branchial) are visible on the dorsal and ventral side of the liver, respectively. The distinct dark blue gallbladder is also visible. The caudal lobe of the liver has been removed to better show the gallbladder. Scale indicates 1 cm.

Fig. 5: The internal structure of the caudal end after the first two incisions. The eggs are obvious as white capsules anchored in the cavity surrounding the intestine. The cloacal gland surrounds the intestine at the cloaca. Scale indicates 1 cm.

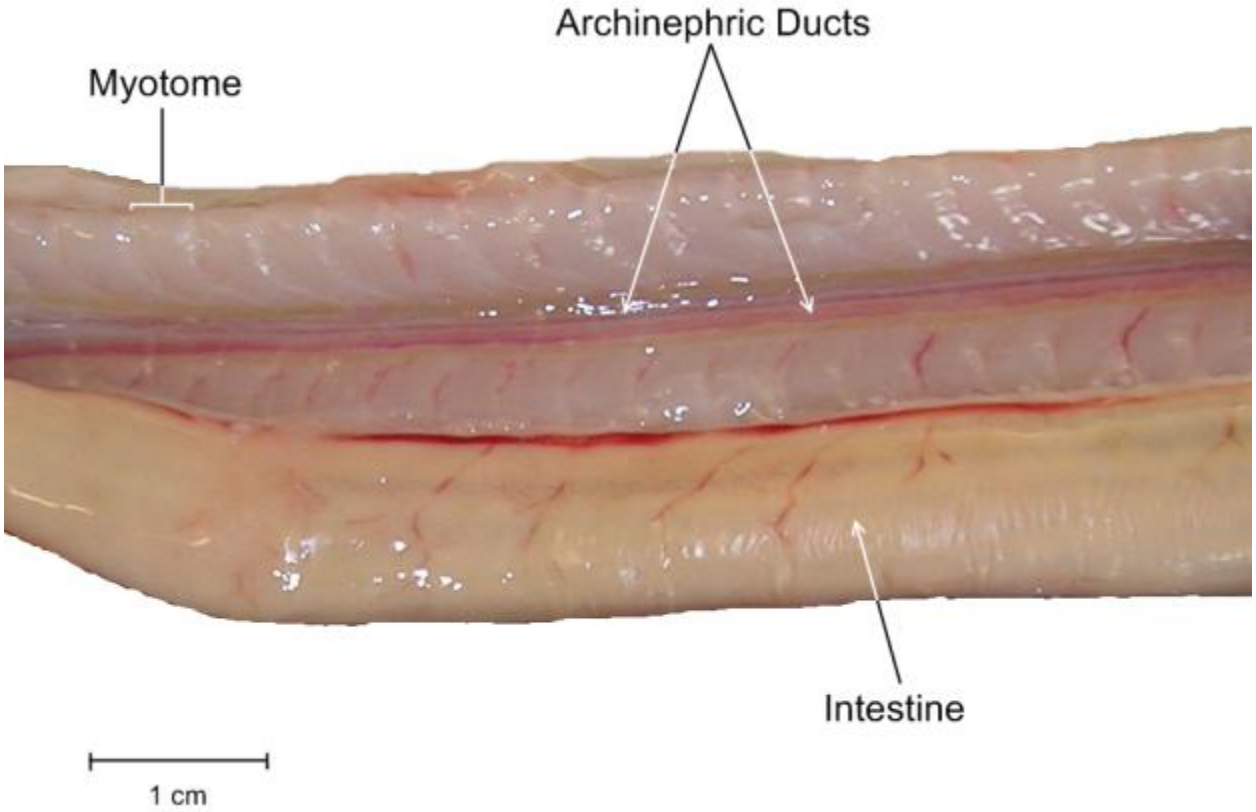

Fig. 6: Displacement of the intestine reveals the two parallel, archinephric ducts of the kidney. A single myotome is also marked. Scale indicates 1 cm.

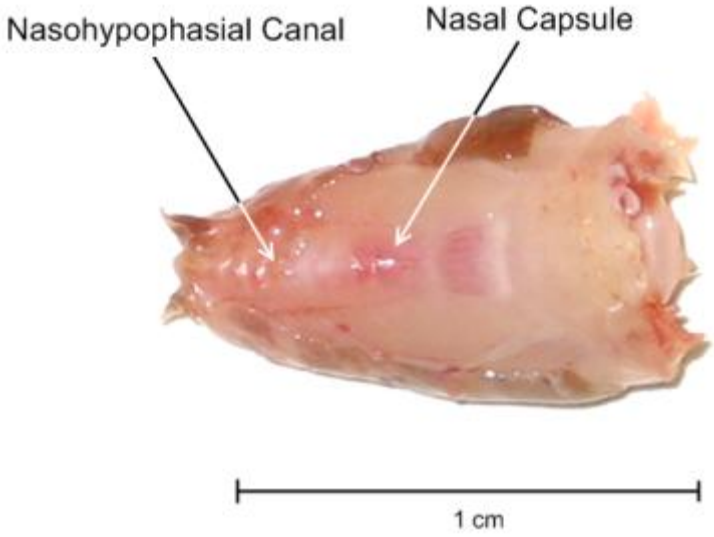

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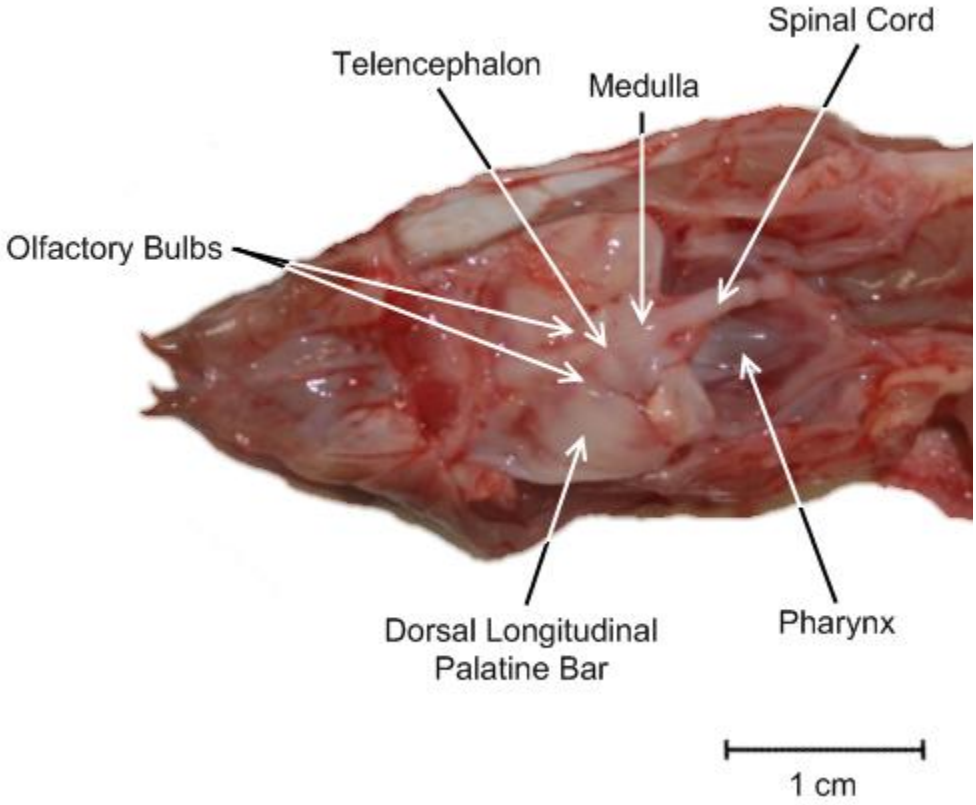
Fig. 7: A dorsal view of the head cartilages revealed after the third incision. The nasohypophasial canal is demarcated by the rippled nasal tube cartilages, and the nasal capsule by the lines of cartilage. Scale indicates 1 cm.



480 **Fig. 8**: A dorsal view of the brain and head cartilages revealed after the fourth and fifth incision. The areas of the brain visible to the naked eye are indicated. The spinal cord is visible exiting the brain posteriorly; the notochord would be found underneath. The brain sits on the dorsal longitudinal palatine bar. The pharynx is visible beneath the dorsal cartilages. Scale indicates 1 cm.

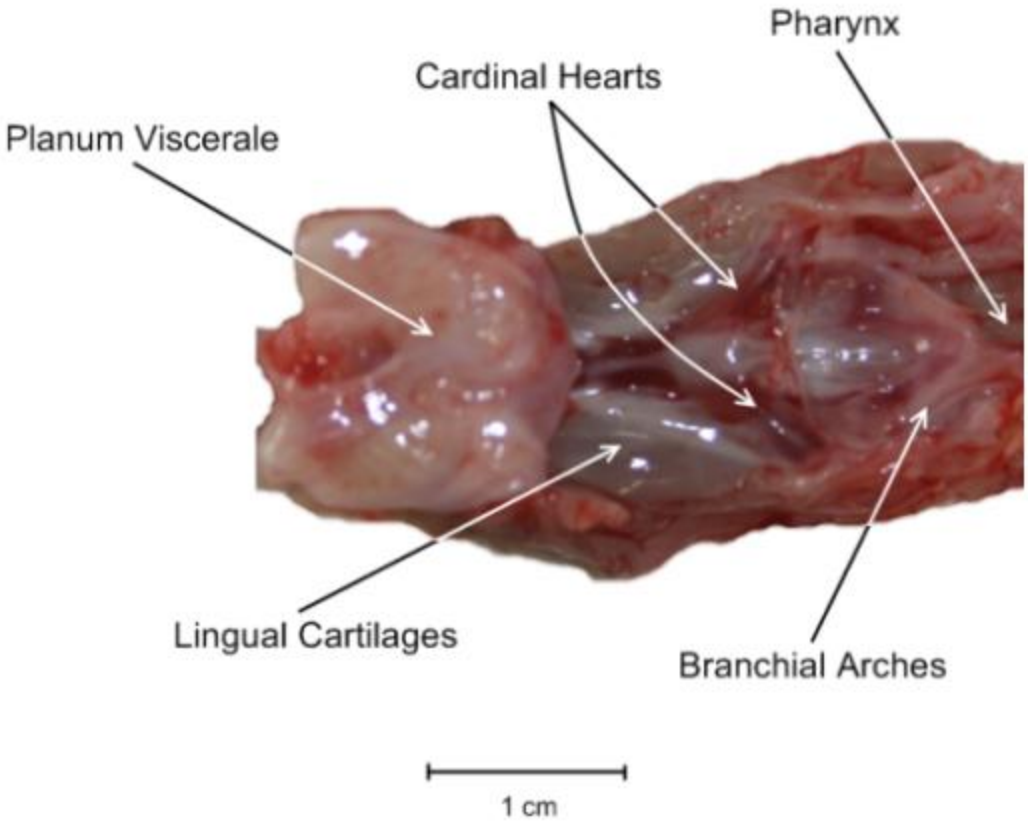


Fig. 9: Lifting up the palatine bar reveals the two cardinal hearts, as well as associated head cartilages. Scale indicates 1 cm.

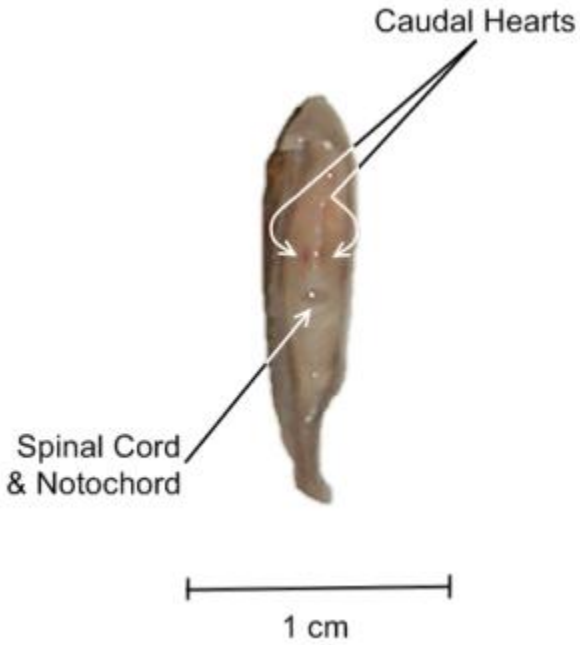


Fig. 10: Frontal section reveals the two caudal hearts at the very tip of the tail, above the spinal cord and notochord. The single finfold can be seen angling off to the right. Scale indicates 1 cm.

ACKNOWLEDGEMENTS

Dr. G. Russell Danner for proposal, review and approval; Dr. Charles Wray at the Mount Desert Island Biological Laboratories for the donation of specimens; Colby College for travel expenses; Joe Slater and Kirby Walker for assistance with photography; Peter Allfather, Jasmine Bruno, Ross Connor, Kimberly Parker, and Jordan Schoonover for manuscript review.

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Serving the World Aquaculture Community

FINQUEL® (MS-222)

Brand of Tricaine Methanesulfonate
For Anesthesia and Tranquilization of Fishes and Other Cold-Blooded Animals

KEEP TIGHTLY CLOSED.
USE ONLY FRESH SOLUTION.

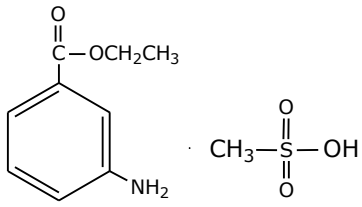
CAUTION: It is imperative to read accompanying descriptive literature before using this drug.
Store at room temperature (Approximately 25° C)

KEEP OUT OF REACH OF CHILDREN

FINQUEL is intended for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals. It has long been recognized as a valuable tool for the proper handling of these animals during manual spawning (fish stripping), weighing, measuring, marking, surgical operations, transport, photography, and research.

WARNINGS
Do not use within 21 days of harvesting fish for food. When used in food fish, use should be restricted to Ictaluridae, Salmonidae, Esocidae, and Percidae and water temperature should exceed 10° C. (50° F.). In other fish and other cold-blooded animals (poikilotherms), FINQUEL should be limited to hatchery or laboratory use.

CHEMISTRY
FINQUEL is the methanesulfonate of meta-amino benzoic acid ethylester, or simply ethyl m-amino benzoate. It is thus an i somer of benzocaine having the formula $C_9H_{11}O_2N + CH_3SO_3H$ and the following structure:



Tricaine Methanesulfonate

FINQUEL is a fine white crystalline powder. Its molecular weight is 261.3. Soluble to 11%, it forms clear, colorless acid solutions in water.

TOXICOLOGY
Comparative toxicological studies carried out on fish and frogs gave the following results:

FISH TOXICITY STUDIES - The toxicity[1] of FINQUEL was measured by standard methods in laboratory bioassays with rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegill, largemouth bass, and walleye. The 24, 48, and 96 hour LC_{50} (lethal concentration for 50 per cent of the animals) values for trout ranged from 52 to 31 mg./liter: for northern pike, from 56 to 48 mg./liter: for catfish, from 66 to 50 mg./liter: for bluegill and largemouth bass, from 61 to 39 mg./liter: for the walleye, the values were 49 to 46 mg./liter.

Safety index: The safety indices for FINQUEL refer to the margin between concentrations which cause anesthesia and mortality. They expressed by the quotient of the lethal concentration for 50 per cent of the fish (LC_{50}) and the effective concentration for 50 per cent of the fish (EC_{50}).

Safety Indices for Rainbow Trout and Channel Catfish at 12°C (54°F):

Species	Exposure Minutes	LC_{50} (mg./liter)	EC_{50} (mg./liter)	Index
Rainbow trout[1]	15	65	32	2.0
"	30	57	32	1.8
"	60	56	29	1.9
Channel catfish[2]	15	139	47	3.0
"	30	118	45	2.6
"	60	110	46	2.4

FROG TOXICITY STUDIES [3] - Frogs were put into various concentrations of FINQUEL for 30 minutes and then transferred to tap water in order to determine the LC_{50}. The LC_{50} was 6.2 per cent FINQUEL. Therefore, the anesthetic must be used in very high concentration before it is fatal to frogs.

DIRECTIONS FOR USE ON FISH
CONCENTRATIONS

FINQUEL is effective and safe for the anesthesia of fish when used as directed. Its use is governed by, and can be tailored to, the needs of individual fishery personnel. Sedation and various rates of anesthetization are controlled by the concentration. The versatility of FINQUEL is demonstrated by the fact that it has been used in fisheries at levels ranging from 10 to 1,000 mg./liter.[3] The action of the anesthetic is slowed at cooler temperatures, in extremely soft water (approximately 10 mg./liter of CaCO3, or less), and in larger fish.[4] Also, efficacy may vary with species.[4] **Thus, it is imperative that preliminary tests of anesthetic to determines the desired rates of anesthesia and exposure times for the specific lots of fish under prevailing conditions.**

The following concentrations may be used as guidelines in selecting concentrations of FINQUEL for the anesthetization of various fishes:

Table 1: Concentration MS-222 Required for Rapid Anesthesia
(Induction time less than 2-5 minutes; used in spawning, marking, measuring, and some surgical operations)

Fish	Temperature	Concentration (mg./liter)	Max. tolerated exposure time* min.)	Recovery time in fresh water (min.)
Salmonidae[4] (Pacific and Atlantic salmon;trout;chars;etc)	7-17° C (45-63° F)	80-135	4-12	3-19
Esocidae[5] (Northern pike; muskellunge)	8-12° C (46-54° F)	150	8-28	8-31
Cyprinidae[3] (Carp; goldfish)	16° C (61° F)	150-200		
Ictaluridae[2] (Channel catfish)	7-27°C (45-81° F)	140-270	4-11	3-24
Centrarchidae[6] (Bluegill;largemouth bass)	10-27° C (50-81° F)	260-330	3-5	7-11
Percidae[5] (Walleye)	10-16° C (50-61° F)	100-120	7-18	5-40
Pet and Tropical[7] Live bearers Egg layers	24-27° C (75-81° F) 24-27° C (75-81° F)	85 75	12 hrs. 12 hrs.	

Table 2: Concentration MS-222 Required for Moderately Rapid Anesthesia
(Induction time less than 15-20 minutes; used in surgical operations and in spawning and marking where longer exposures are more important than rapid immobilization)

Fish	Temperature	Concentration (mg./liter)	Max. tolerated exposure time (min.)	Recovery time in fresh water (min.)
Salmonidae[4] (Pacific and Atlantic salmon;trout;chars;etc..)	7-17° C (45-63° F)	50-60	30 or >	2-20
Ictaluridae[2] (Channel catfish)	7-27°C (45-81° F)	70	30 or >	1-10

Table 3: Concentration MS-222 Required for Sedation
(Induction within 15 minutes; used in fish transport)

Fish	Temperature	Concentration (mg./liter)	Maintenance of sedation (hr.)
Salmonidae[4] (Pacific and Atlantic salmon;trout;chars;etc..)	7-17° C (45-63° F)	15-30	6
Esocidae[5] (Chain pickerel)	8-12° C (46-54° F)	40	
Ictaluridae[2] (Channel catfish)	7-27°C (45-81° F)	20-40	6
Centrarchidae[6] (Bluegills)	10-27° C (50-81° F)	25	8-13
Pet and Tropical[7] [Bettas, Piranhas, etc. (uncrowded)	24-27° C (75-81° F)	66	48
Goldfish]	24-27° C (75-81° F)	37	48

IMPORTANT: Since, in many cases, relatively rapid rates of anesthesia can be achieved only by exceeding the lethal concentration of FINQUEL, it is necessary to return anesthetized fish to fresh water before they are overexposed. Excessive exposures are avoided by observing the following sensory and motor responses of the fish which characterize progressively deeper levels of anesthesia.

Sedation - Decreased reactivity to visual and vibrational stimuli; opercular activity reduced.

Total loss of equilibrium - Fish turns over; locomotion increases; fish swims or extends fins in response to pressure on caudal fin or peduncle.

Total loss of reflex - No response to pressure on caudal fin or peduncle; opercular rate slow and erratic.

Medullary collapse - Opercular activity ceases.

Laboratory and field investigations, [3,9] have shown that the action of FINQUEL is readily reversed when the fish are transferred to fresh water before opercular activity ceases. *Additional exposure following medullary collapse may result in mortality.* A rough estimate of the safe total exposure can be made by multiplying the time required for anesthesia by a factor of 2 or 3.

WATER
Since FINQUEL is very soluble (1:9) in water, it dissolves with equal readiness in spring water, tap water, or seawater. *Do not use distilled or deionized water, or water containing chlorine. Heavy metals (copper, zinc, etc.), or other toxic contaminants.* The anesthetic solution should be well oxygenated, and its temperature should be similar to that of the water from which fish are taken. In the field, many water quality problems are eliminated by using natural water to which the fish are acclimated, provided the water does not possess high chemical or biologic oxygen demand.

METHOD OF APPLICATION

1. General anesthesia: - For most situations where rapid or moderately rapid anesthesia is required, FINQUEL may be applied in a bath, i.e., the fish are immersed in the anesthetic solution. Containers may be of glass, plastic, steel, aluminum, or other suitable material. *However, do not use galvanized or brass containers unless treated or sealed to prevent dissolution of zinc.* Size of container is determined by individual needs, but the fish should not be overcrowded. Discard anesthetic solutions when a loss in potency is noted, or when the solutions become fouled with mucus or excrement.

2. For surgery and certain physiologic studies, the fish may be anesthetized to loss of reflex, removed from the anesthetic, and then positioned so that the gills are bathed in a sedating concentration of FINQUEL. Some investigators have developed flowing, recirculating systems for bathing the gills with anesthetic during surgery.

Large fishes such as sharks and rays are anesthetized within minutes by spraying the gills with a 1g./liter solution of FINQUEL.[10] The application is made by means of a water pistol, bulb syringe, hand pump, etc.

3. Transport - FINQUEL has been used to sedate fish during transport. It is more successful in cold than in warm water, and it is instrumental in reducing injuries because of hyperactivity. Fish are usually transported by means of distribution units (tank trucks), or by air in plastic bags.[11,12] In either case, the fish should be fasted before-hand to reduce metabolic wastes. Also, some workers suggest pretransport sedation for several hours to lower metabolism. With distribution units, the fish may fasted and sedated prior to loading. The anesthetic solution is prepared in the distribution unit and oxygenated. Then, the fish are added and temperature acclimated.

In air shipments, the anesthetic solution is placed in a suitable plastic bag, the sedated fish are added, the bag inflated with oxygen, tied securely, and placed in a second bag. This bag is also tied, and then placed on ice in an insulated container.[13] A modification of this method involves complete anesthesia of the fish, and placing them in water bags which contain no anesthetic. In any case, upon arrival, the fish should be acclimated slowly to new environmental temperatures.

PREPARATION OF FINQUEL® SOLUTIONS

Prior to use, FINQUEL may be weighed out into amounts which are convenient for the volume of water to be used. A handy units is 2 g. since this quantity in 5 gallons of water yields a concentration of about 100 mg./liter. For rough approximations, one level teaspoonful contains 2.0 to 2.5 g. Thus a level teaspoonful of anesthetic in 5 gallons gives a concentration of about 120 mg./liter.

To convert mg./liter into g./gal.: multiply number of mg. by 0.00378
e.g. 80 mg./liter = 80 x 0.00378 = .0302 g./gal.

To convert mg./liter into a ratio of FINQUEL to water: divide 1,000,000 by the number of mg.
e.g. 80 mg./liter = 1,000,000 / 80 = 1:12,500

LIMITATIONS IN USE

Since FINQUEL is taken up into the blood of fish, residues of the drug may occur in edible tissues. However, the residues dissipate rapidly after the fish are placed in fresh water.[14] Thus*, treated fish which may be used for food must be held in fresh water above 10°C. (50°F.) for a period of 21 days.*

Withdrawal in fresh water is unnecessary for non food fishes such as goldfish, bait fish, and ornamentals. Also, withdrawal is unnecessary for sublegal sizes of the following species of fish because they are not used as food immediately following anesthesia (Table 4).

Table 4 - Sublegal Sizes of Fish Species not used as Food Immediately after Anesthesia[15]

Species	Size (in.)	Species	Size (in.)
Pink salmon	6	Lake trout	5
Chum salmon	6	Splake trout	6
Coho Salmon	6	Grayling	6
Sockeye salmon	6	Northern pike	12
Chinook Salmon	6	Muskellunge	12
Cutthroat trout	6	Channel catfish	6
Steelhead trout	8	Flathead catfish	6
Rainbow trout	6	Bluegill	3
Atlantic salmon	10	Redear sunfish	3
Brown trout	6	Smallmouth bass	5
Brook trout	6	Largemouth bass	5
		Walleye	6

PRECAUTIONS

1. Avoid inhaling FINQUEL or getting it into the eyes.
2. Always conduct preliminary tests with FINQUEL to determine desired rates of anesthesia and optimal length of exposure.
3. Do not overexpose fish to lethal levels of FINQUEL.
4. Do not anesthetize more fish than can be handled effectively.
5. Do not contaminate eggs or sperm with FINQUEL when stripping fish.
6. Do note use water containing chlorine, or other toxic agents.
7. Insure adequate oxygen in anesthetic solution.
8. Discard anesthetic solutions when fouled with mucus or metabolic wastes.
9. Do not discard FINQUEL solutions into water supplies of natural waters.
10. Store FINQUEL solutions in a cool place away from light.*
11. Discard stock solutions of FINQUEL after several days.*
12. Treated fish destined for food must be held in fresh water above 10°C. (50°F.) For 21 days before use.

*The color of FINQUEL solutions may change rapidly to yellow or brown when exposed to light. This does not affect activity in any significant way. However, for best results use freshly prepared solutions. A 10 per cent solution stored at room temperature shows no significant loss of potency after three days, but after 10 days, a brownish color and an activity decrease of about 5 per cent is observed.

II. GUIDELINES FOR USE ON AMPHIBIANS

Table 5. Effects of Varying Concentrations of FINQUEL® on Salamanders

Salamander	Concentration *	Duration of Anesthesia *	Remarks
EMBRYOS	1:10,000 (3b)	2 days	No adverse effects
Ambystoma opacum	1:3,000 (3c)	To 30 min.	"
LARVAE	1:1,0000 (3b)	2 days	"
	1:12,000(3f)	10-15 min.	
	1:20,000(3f)	10-15 min.	"
Ambystoma opacum	1:3,000 (3c)	To 30 min.	"
ADULTS	1:1,000 (3b)	Few min.	"
	1:3,000 (3b)	3 day	"
Newts	1:1,000 (3b)	Few min.	"
	1:10,000 (3b)	2 days	"
Triturus sp.	1:1,000 (3k)	20 min.	"
Triturus uridescens	1:3,000 (3g)	1 hour	"
Mole salamanders *Ambystoma opacum*	1:3,000 (3c)	To 30 min.	"
Ambystoma tigrinum	1:2,000 (3j)		"
Ambystoma punctatum	1:2,000 (3j)	15-30 min.	"
Mud-puppy *Necturus maculosus*	1:1,500 (3i)	To 6 hours	Maintenance does, 0.1 of induction concentration. At exposure to induction concentration of more than 20-30 min., renal circulation becomes sluggish or stops.

*When an individual of any of the species listed is exposed at the designated concentration, the data available suggest that the animal may be safely maintained under anesthesia for the time noted. Prolonging exposure to the anesthetic beyond the time indicated may cause deaths. See PRECAUTIONS.

Table 6 - Effects of Varying Concentrations of FINQUEL® on Frogs

Frog	Concentration*	Duration of Anesthesia *	Remarks
EMBRYOS	1:1,000 (3b)	few min.	No adverse effects
	1:10,000 (3b)	2 days	"
	1:15,0000 (3h)	3 days	"
TADPOLES	1:1,000(3j)	30 min.	No adverse effects
	1:3,000(3f)	10-15 min.	"
	1:10,000 (3b)	2 days	"
	1:15,000 (3h)	3 days	"
Rana sp.	1:5,000 (3k)	5 hours	No adverse effects
Rana pipiens	1:1,000 (3j)	15-30 min.	"
	1:3,333 (3a)	2 min.	"
	variable (3d)	1 hour	"
ADULTS	1:1,000 (3c)	30 min.	No adverse effects
Leopard frog *Rana pipiens*	1:3,000 (3c)	to 30 min.	No adverse effects
Eastern wood frog *Rana sylvatica*	1:8,000 (3j)	5-10 min.	Only slightly under anesthesia.

*When an individual of any of the species listed is exposed at the designated concentration, the data available suggest that the animal may be safely maintained under anesthesia for the time noted. Prolonging exposure to the anesthetic beyond the time indicated may cause deaths. See PRECAUTIONS.

AVAILABILITY OF FINQUEL®

Bottles of Net Wt. 0.18 oz. (5 grams) Net Wt. 3.5 oz. (100 grams), and Net Wt. 2.2 lb. (1000 grams of tricaine methanesulfonate.

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(Finquel® MS-222 Package Insert for NADA 042-427)