Eggs, larvae and adults of the four subspecies of *Hyla regilla* occurring in Oregon as given by Jameson, Mackey and Richmond (1966) were collected and subjected to salt stress in a series of graded seawaters.

It was found that in all cases adults were more tolerant than larvae and larvae were more tolerant than eggs. It was also found that animals in all stages taken from the area extending westward from the crest of the Oregon Coast Range to the sea, designated as the range of *Hyla regilla pacifica*, were more tolerant of elevated salt levels in their ambient medium than animals from the three other subspecies areas in Oregon.

Observations were made of the effect of elevated salinities on the eggs and the development of the embryos with respect to the vitelline membranes, the closure of the blastopore and neurulation.

Mechanisms were postulated to explain the observed differences in tolerance to salt stress.
Variations in Salinity Tolerance in the Pacific Treefrog, Hyla regilla, in Oregon

by

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VARIATIONS IN SALINITY TOLERANCE IN THE PACIFIC TREEFROG, *Hyla regilla*, IN OREGON

INTRODUCTION

The Pacific Treefrog, *Hyla regilla*, is one of the most ubiquitous amphibians in the Pacific Northwest. Its range includes the states of Washington and Oregon, most of California and Nevada, parts of Idaho, Montana and British Columbia, and even includes a disjunct population on the southern tip of Baja California. Most range maps include the furthest limit of a population and assume that the reader realizes that there may be large areas within the range that for one reason or another are unsuited as habitat for the animal in question. Of course this sort of reasoning applies to *Hyla regilla* but to a much lesser degree than to most animals.

*Hyla regilla* is a remarkably terrestrial amphibian and is found at rather considerable distances from water. When it does seek water, it is almost invariably in small pools, quiet shallow backwaters, roadside ditches, or even seeps. These bodies of water can be surprisingly small and still be acceptable to *Hyla*, both as a source of water for hydration and as a breeding site. One of the smallest pools to be reported in this paper was a seep in the Coast Range that fed a small roadside pool 50 mm deep, 150 to 200 mm wide and 1.5 to 2 meters long. Despite the small size, the pool
contained hundreds of tadpoles during the latter part of May.

The variety of breeding habitats in which these animals occur is remarkable. These extend to the edge of the sea in rocky depressions well within the spray zone. One population sampled in this study came from a "freshwater" pool within 5 meters of mean high tide and the tadpoles and eggs were collected in a shower of salt spray. The animals are equally at home in freshwater pools and ditches in the Corvallis area, in pools of snowmelt along the crest of the Oregon Cascades, in small springs in the ponderosa pine region to the east of the Cascades and far out in the sagebrush country where a creek, spring, or even a stock tank provides a reliable source of water.

In areas having a mild climate *Hyla regilla* has a remarkably long breeding season compared to other anurans. In the Corvallis area where winter nighttime temperatures rarely fall below freezing and often run as high as 10°C the breeding season extends from December or January to April or May. On the coast in the area from Depoe Bay south to Neptune State Park breeding activity usually begins about the first week of March and extends well into June and I have found tadpoles as late as July or even the first of August. In areas with more extreme climatic restrictions such as the High Cascades where snow remains until June and cold weather may
return in September, or out on the high desert where seasons are also short and the water supply is uncertain late in the summer, there is a short intensive burst of breeding activity that lasts a week or two at the most. This ensures that the eggs and tadpoles will have an optimum opportunity to mature.

As is the case with most anurans the males come to the breeding pools first and begin to call or "croak." The females who are by now heavy with eggs come to the pools later, possibly attracted by the calling of the males. Upon entering the water the females swim about until they touch a male and are grasped by the male just behind the front legs. In the ensuing mating the eggs are deposited in several small packets attached to vegetation growing in the pool. After the eggs are shed the females leave the pool while the males stay for extended periods of time and may mate with several females.

The development of the eggs follows the normal anuran pattern, resulting in free-swimming tadpoles in a few days or weeks at most. Generally speaking maturation is faster at temperatures near 20°C and progressively slower at lower temperatures. When the tadpoles emerge they face a very hostile environment with many predators, ranging from predaceous water insects to fish and other amphibians, both larval and adult, snakes, shorebirds etc. They also face the hazard of having the pool in which they were hatched drying up. Even though those young that survive use every bit of available cover the
attrition is high and the chances of any one egg becoming an adult animal are very low. Nevertheless, the eggs are produced in tremendous numbers and the species thrives despite individual losses.

*Hyla regilla* was first described by Baird and Girard (1852) from material collected by an expedition under the command of Capt. Charles Wilkes, USN. However these early workers failed to designate a type specimen. Test (1898) referred to Baird and Girard's work and listed the specimens they had described as type specimens in the belief that they had been so designated. Several other workers have examined this early material and now there is general agreement that USNM, #9182, collected near Ft. Vancouver, Washington, is the true type specimen of *Hyla regilla*.

Jameson, Mackey, and Richmond (1966) in their paper on the systematics of *Hyla regilla* have divided the species occurring in Oregon into four subspecies based on variations in their external morphology. The parameters used in making these divisions are as follows: snout-vent length, shank length, forearm length, length of the fourth finger, head length, eye to tympanum length, eye to nostril length, head width, face height and area of the toepad. The subspecies occurring in Oregon, based on the foregoing measurements are as follows:

1. *Hyla regilla pacifica* found from the ridge of the Oregon Coast Range westward to the edge of the sea.
2. *Hyla regilla regilla* occurring in the Rogue, Umpqua, and Willamette Valleys and extending from the ridge of the Oregon Coast Range eastward to the foothills of the Cascade Mountains.

3. *Hyla regilla cascadae* largely restricted to the higher elevations of the Cascade Mountains.

4. *Hyla regilla palouse* in the area east of the Cascade Mountains, including several mountain ranges and the sagebrush area generally referred to as the Oregon Desert.

It should be pointed out that Jameson, Mackey, and Richmond (1966) established the described subspecies on the basis of average measurements taken on a large number of individuals and it is very difficult to place a single individual in any one subspecies. Nevertheless the geographical areas in Oregon designated for the various subspecies of *Hyla regilla* in their paper have been used as a basis for collecting the animals used in this study.

Brown (1969) and Brattstrom and Warren (1955) note that an animal breeding in shallow temporary pools will be subject to wide temperature fluctuations due to the rapid warming and cooling of such small bodies of water. Even with its high thermal capacity the small amount of water in these pools cannot resist the effects of bright sunlight or a cold clear night. The water in one of the pools in the canyon of the South Fork of the Walla Walla River in which collections
for this work were made read 2°C at 6:30 AM and 20°C at 11:00. The highest temperature found was 33°C for a pool from which samples were taken near Stinkwater Pass east of Burns, Oregon.

Brattstrom and Warren (1955) report 10°C as the lowest temperature at which *Hyla regilla* will call, and that the frogs stopped calling and sought cooler water when the ambient temperature reached 20°C. Cunningham and Mullally (1956) reported a California population of *Hyla regilla* that called in water down to 7°C and did not stop calling entirely until the temperature fell to 3.8°C. The same authors report that *Hyla regilla* are capable of slight movement when the water temperature has fallen to 0.2°C. Brattstrom and Warren (1955) report that optimum breeding temperatures lie between 12°C and 15°C and other workers are in substantial agreement with these figures. The same authors reported that eggs in neurula and tail bud stages could survive temperatures of -5°C to -7°C for two hours while Sechtman and Olson (1941) reported normal development, upon warming, when embryos from blastula to tail bud had been frozen in ice at -5°C to -7°C for eight days.

Sechtman and Olson (1941), Brattstrom and Warren (1955), and Cunningham and Mullally (1956) all report normal development of tadpoles up to 33°C. Increasing mortality and malformations were reported from 33°C to 38°C and 100% mortality occurred above 38°C. All authors seem to agree that 38°C is the upper lethal limit for both
adult and larval *Hyla regilla* and Brattstorm and Warren (1955) note that while the foregoing temperatures represent thermal maxima for the species the tadpoles prefer $20^\circ C$ while the adults prefer $15^\circ C$. Moore (1939) notes that while amphibian eggs may develop normally over a rather wide range of temperatures, optimal development occurs near the preferred temperatures.

Obviously an animal that can withstand wide fluctuations in a vital factor affecting its survival will occur in a much wider range than an animal that lacks this plasticity. By the same reasoning the animals that occupy that part of the range where conditions approach critical limits will have a much higher proportion of reproductive failures than those in more favorable locations. Livezey (1953) reported a population of *Hyla regilla* at 2194 to 2521 meters in the Sierra Nevada of Central California that had been held back by unusually severe weather and were calling on July 16, 1952. It would take optimal temperature and water conditions to ensure that the eggs from these matings would reach adulthood before the return of cold weather in the fall, and it is possible that none of this particular group of eggs would develop into a mature animal.

While it has been known for a long time that amphibians and their eggs and larvae in particular are susceptible to elevated salt levels in the water in which they occur, there have also been persistent reports of amphibians living and breeding in estuarine habitats
and brackish pools associated with seaside sand dunes, mineral springs, and desert streams. Hardy (1943) in a brief note reported four British amphibians breeding in brackish water on the coast of Lancashire. Peterson, Garrett, and Lantz (1952) found *Hyla dominicensis*, *Hyla cinera*, and *Microhyla carolinensis* breeding in brackish water on the Florida Keys. Unfortunately neither of these papers mention the exact salinity of the water in which the animals were found.

Ackrill, Hornby, and Thomas (1969) working with *Rana temporaria* and *Rana esculenta* found that under laboratory conditions both species could tolerate 7% salt in their ambient medium for at least two months, and perhaps indefinitely. Spurway (1943) found newt larvae presumed to be *Molge palmata* living in a layered pool on the English Coast. The upper portion of the pool where the newts stayed had a salt concentration of 1.8% while the lower layer of the pool where they rarely ventured had a concentration of 19.2%. Ruibal (1962b) working in the dry areas of Northwestern Argentina found five species of leptodactylid frogs and one species of *Bufo* living in water that ranged from 1% to 10%. Ely (1944) found that *Bufo marinus* larvae would develop normally in 4.8% salt concentrations, but that virtually 100% mortality was achieved shortly after hatching if the salt concentration was elevated to 6.4%. Taylor (1943) collected *Ambystoma subsalsum* in a brackish lake in Mexico with a salt
concentration of 8%. Ruibal (1959) working with a population of Rana pipiens inhabiting a small creek in Southern California running into the Salton Sea found a range of tolerance of 6% to 13% in adults depending on where the frogs were collected in the stream. The corresponding levels for the eggs were 3.8% and 5%. Gordon (1962) reported that the European green toad, Bufo viridis, occurred regularly in water with a salt concentration of 20% and under laboratory conditions was able to tolerate 22% to 29% depending on the location in which the animals were collected. Data on the collecting sites were not supplied, but it is supposed that most of these animals came from the shores of the Baltic Sea. The same author reported another study with Bufo viridis where toads from two separate areas were able to tolerate 19% and 23% respectively, but that if the animals were given a free choice they gathered in water with a salinity of 8%. Gordon, Schmidt-Nielsen, and Kelley (1961) reported that the crab-eating frog, Rana cancrivora from the Gulf of Siam could tolerate a salt concentration of 28% and surprisingly that the tadpoles could tolerate 39% or 120% seawater! This tolerance of seawater accords well with their habitat and natural history, as they live in holes under the knees and roots of tidewater mangroves and emerge to feed on the small crabs that become active on the incoming tide.

Any animal will lose water when exposed to heat and dry air. Chew and Dammann (1961) working with small reptiles, mammals,
and anurans inhabiting deserts found that these animals normally lose water in a ratio of 1:10:40 respectively. These animals were subjected to about the same stresses and hence lost comparable amounts of moisture by respiration suggesting that the mammals and anurans had to lose the larger amounts of moisture from some other area of their bodies. These authors were able to show that a reptile's skin is very nearly "waterproof," and since excreted water was also accounted for in the foregoing ratio the evidence points strongly to the skin of the mammals and anurans as the area from which the water was lost. Adolph (1932) and Thorson (1955) showed that amphibians lose water freely through their skins. Adolph (1931) and Thorson (1956) both showed that the rates of desiccation of dead frogs were about the same for the animals that were left intact as they were for animals that had been skinned, but that a living frog could exert a measure of control over the rate at which water was lost.

One obvious way for a desiccated amphibian to rehydrate is to immerse itself in water. In this situation McClanahan (1967) has shown that adult amphibians don't drink, but rehydrate themselves by taking water through their skins. It is thus seen that water may pass freely through an amphibian's skin in either direction. The fact is that many desert dwelling amphibians don't have bodies of water available and Packer (1963), Dole (1967), and Lee (1968) have
shown that anurans can rehydrate by sitting on moist soil, or less importantly on dew covered vegetation. Dole (1967) has further shown that partially desiccated *Rana pipiens* can rehydrate themselves in 48 hours if the soil contains 20% moisture while they can regain 50% of their weight loss in 48 hours on soil containing as little as 10% moisture. Lasiewski and Bartholomew (1969) have proposed that while desert air temperatures fluctuate widely, the temperatures in the burrows inhabited by poikilothermic amphibians remain fairly constant. Thus an amphibian cooled by the desert night air during its foraging would, on returning to its relatively warm burrow, lower the dewpoint sufficiently to collect condensation on its body which could be absorbed through the skin. It remains to be shown that this mechanism is important in the hydration of these animals, but it is an interesting possibility.

Ray (1958), Mayhew (1965) and Bently (1966) have all shown that desiccated animals have a higher blood electrolyte concentration than hydrated forms, and Tercafs and Schoffeniels (1962) suggest that this elevated blood electrolyte level may well be a gradient along which hydration will proceed. Shoemaker (1965) found that if he loaded toads with osmotically equal amounts of NaCl, sucrose, and urea, the NaCl and sucrose stimulated hydration while the urea had no effect, supporting the suggestion that elevated blood electrolytes will stimulate water uptake. Claussen (1969) found that there was
no correlation between the rate at which an amphibian lost water and the habitat in which it was found, but that there is a correlation between an animal's ability to withstand desiccation and the moisture level of the habitat in which it is found. Schmid (1965) working with nine species of amphibians reported the same relationship, and also found that the blood of normally hydrated aquatic forms was more dilute than the blood of normally hydrated terrestrial forms. He also noted that terrestrial forms tend to rehydrate faster than aquatic forms, and that these animals seem to have less resistance to hydrostatic stress than aquatic forms. Ray (1958) found that salamanders follow the same pattern and can tolerate water losses at 7% to 50% of their body weight depending on the species. He also noted that the females tended to tolerate a greater water loss than the males, but this may have been due to the water associated with the egg mass of gravid females forming a larger reservoir from which to draw. In contrast to Schmid (1965) and Claussen (1969), Ray (1958) reported that in the case of salamanders, at least, those from the more xeric habitats could slow their evaporative water loss to a greater extent than animals from a more mesic habitat. He suggested that in these animals the water loss may be controlled by some mechanism acting in the deep layers of the skin. Khalil and Abdel-Messieh (1954) found a similar relationship between the tissue fluid levels of small mammals and reptiles living in the desert and those of similar animals living in less arid areas. Bentley, Bretz and Schmidt-Nielsen (1967) found that the diamond backed terrapin, *Malaclemys terrapin*
centrata, which lives in an estuarine habitat can survive for some time in seawater, but that it gradually undergoes desiccation in the form of an increasing Na$^+$ load with a corresponding reduction in body water. This loss of body water is rapidly reversed when the animal is returned to fresh water. These workers also showed that an animal undergoing salt stress has to expend energy in its attempt to maintain osmotic integrity as evidenced by an increased oxygen consumption. Thesleff and Schmidt-Nielsen (1962) working with Rana cancrivora which inhabits an "aquatic, osmotically xeric habitat" found that single muscle preparations would tolerate twice the electrolyte concentrations in the bathing medium that a similar preparation from a freshwater frog, Rana pipiens, could tolerate; provided that 250 mM urea was present or that the solution was aerated. If these conditions were met contractions still occurred until the electrolyte level rose to three to four times the normal level.

McClanahan (1964) in a paper comparing Bufo cognatus and Scaphiopus couchi with Rana pipiens found that water loss and the concentration of inorganic ions were the factors most critically affecting muscle contraction.

Hall (1922) working with Rana pipiens and Ambystoma punctatum showed that a lethal water loss was between 10.5% and 11.3% of the body weight in the ranid and 37% to 44% for the ambystomatid.

Littleford, Keller and Phillips (1947) working with four Eastern
American plethodontid salamanders found that these animals could recover after losing 18% of their body weight by desiccation and that their vital limit with respect to weight loss due to desiccation was about 25%. Packer (1963) working with the desert dwelling Australian frog *Heleioporus eyrei* found that death occurred when the animals were desiccated by 40.7% of their fully hydrated weight. One of the most xeric of all amphibians is the spadefoot toad, *Scaphiopus couchi*. McClanahan (1967) found that the animals could lose 47% of their body weight by desiccation before they could not recover, and that if the animals were directly desiccated, death did not occur until they had lost 60% of their body weight. In this connection, there is general agreement that most amphibians have a body water content of about 80%. Mayhew (1965) found that blood electrolytes of emerging *Scaphiopus couchi* ran as high as 20‰ compared with 7-8‰ in active animals. While moisture is extremely important in breaking aestivation in these forms, Martin (1969) working with the Australian burrowing frog, *Limnodynastes dorsalis*, and Mayhew (1965) working with *Scaphiopus couchi* have demonstrated a critical temperature requirement as well, implying that metabolic work must be done to reduce the salt content of the animal immediately on arousal.

Hydrated anurans have comparatively large amounts of water in their urinary bladders which can be resorbed in response to desiccation. Shoemaker (1964) found that adult *Bufo marinus* use dilute
urine to protect themselves against dehydration and Claussen (1969) reports that bladder water will slow the desiccation of the entire animal, but in his opinion it is doubtful if this mechanism has any long range adaptive significance. Lee (1968) found that as long as water remained in the bladders of *Heleioporus eyrei* there was no rise in the osmolarity or in the Na\(^+\) content of the animal's blood. Ruibal (1962), working with the toad *Bufo cognatus*, showed that the bladder contents of a fully hydrated animal account for 19% to 31% of its gross weight. In these same frogs the salt concentration of the urine of a hydrated animal was 1% while the blood and lymph ranged from 6% to 7%. Animals with full bladders had remarkably stable electrolyte levels, and even if as much as half the urine was lost and the salt concentration of the remaining urine doubled there was essentially no change in the blood electrolyte levels. McClanahan (1967) found that in *Scaphiopus couchi* the plasma and urine concentrations were about the same in the emerging animal, while in a fully hydrated animal the plasma concentration is about six times as great as that of the urine.

The most common method of ridding an amphibian's body of its excess salts is by renal clearance. Adolph (1927) found a close correlation between the concentration of the ambient medium and the amount of urine produced. He found that a change of 0.1M NaCl in the ambient medium was sufficient to change the rate of excretion.
In addition some animals have specialized organs or tissues for the excretion of excess salt. Bently, Bretz, and Schmidt-Nielsen (1967) found that *Malaclemys terrapin centrata* has an orbital salt gland, and both Copeland (1950) and Doyle and Gorecki (1961) found evidence of chloride cells in the gills of salt water fish.

It has been recognized for a long time that while an amphibian can't resist desiccation for a long period of time there is some mechanism that slows the loss of water through the skin. Adolph (1931) suggested that a mechanism under nervous control hindered the free movement of water through the skin of a living amphibian. Thorson (1965) showed that living frogs can resist desiccation to some degree, but once they are dead desiccation progresses rapidly and there is no difference in the rate in dead animals whether they are skinned or intact. Shoemaker (1965) showed that amphibians respond to desiccation by reducing urine output and increasing water uptake through their skin. He was further able to show that this mechanism was under the control of a hormone similar to antidiuretic hormone in mammals. Jasinski and Gorbman (1967) demonstrated the presence of this antidiuretic hormone in the tissues of badly desiccated animals by histochemical means, indicating that large amounts of hormone are elaborated in response to increasing stress. Ewer (1950) found that injections of pituitrin would both shut down renal function and increase the uptake of water through amphibian
skin. Ewer (1952a) found further that pituitrin would uniformly load an animal in an aquatic situation, but that in a dry situation fluid is taken from the rest of the body and stored in the dorsal lymph sacs. Ewer (1952b) compared the effects of the pituitary secretions pitocin and pitressin on the xeric adapted anurans Bufo regularis and Bufo carens with their effects on the aquatic frog Xenopus laevis. He found that the toads were most sensitive to pitressin and that they responded by increasing the water uptake through their skin and by exhibiting anti diuresis. Xenopus, on the other hand, responded equally to both pitocin and pitressin with respect to water loading, but exhibited no antidiuresis. He found further evidence of habitat adaptation in these species in the fact that if all three species were desiccated equally, both of the Bufos had much higher loading rates than the aquatic Xenopus.

Ackrill, Hornby and Thomas (1969) found that if most frogs were placed in a hypertonic ambient medium their kidneys were unable to produce urine hypertonic to their body fluids, and that they tended to raise their blood electrolytes to compensate for the rise in the salt concentration in their environment. Schmidt-Nielsen and Lee (1962) reported that Rana cancrivora from the Gulf of Siam regularly seek food and swim in full strength sea water and that this behavior was accompanied by a twofold rise in the salt concentrations of body fluids and a high concentration of urea (about 0.3M). Gordon,
Schmidt-Nielsen, and Kelley (1961) found that while the kidneys of most freshwater frogs had comparatively few active glomeruli, the kidneys of *Rana cancrivora* had nearly all their glomeruli well developed, suggesting that in addition to surviving elevated blood and body fluid salt and urea levels the kidneys of these animals are able to produce more concentrated urine than those of most other amphibians.

Munro (1953) has shown that in most cases tadpoles tend to excrete waste nitrogen as ammonia, but as they metamorphose there is a sharp drop in ammonia excretion correlated with a spectacular rise in urea excretion. In the free swimming tadpoles 80% to 90% of the nitrogen is excreted as ammonia and 10% to 20% as urea. During tail absorption the percent of nitrogen excreted as ammonia falls from 65% to 20% in five days. Since ammonia is considerably more irritating to animal tissue than urea, the advantage of excreting ammonia in the aquatic stage and urea in the situation where less water is available becomes obvious, and indeed Munro (1953) points out that the highly aquatic frog *Xenopus laevis* excretes 80% of its nitrogen as ammonia even in the adult stage. Balinsky, Cragg, and Baldwin (1961) were able to show that even *Xenopus* can switch to urea excretion under stress conditions and revert to ammonia excretion when it gets back into a normal aquatic habitat. The frog will also store waste nitrogen as urea thus providing a comparatively non-irritating substance to raise its body's osmotic concentration in response to high salt levels in the ambient medium. This urea will be excreted rapidly when the animal is returned to freshwater.
Tercafs and Schoffeniels (1962) showed that many amphibians use this mechanism to raise the concentration of their body fluids in response to external stress and suggest that this is an osmoregulatory phenomenon rather than a detoxification mechanism. McClanahan (1964) is in agreement with this concept and has also shown that muscle is much more tolerant of urea than other substances such as sucrose and NaCl. Cragg, Balinsky, and Baldwin (1961) point out that aquatic forms generally excrete more of their waste nitrogen as ammonia than urea, while damp dwelling terrestrial forms excrete more urea than ammonia, and forms subject to extreme desiccation tend to excrete uric acid. Since it takes about twice the energy to form a mole of urea and about six times as much energy to form a mole of uric acid as it does to form a mole of ammonia it can be inferred that for the excretion of nitrogen as urea or uric acid to persist it must confer some survival advantage to the organism possessing this ability. It seems more than likely that the advantage conferred is the ability to raise body electrolyte levels in response to external stress with relatively non-irritating substances.

Since adult frogs almost always hydrate themselves by absorbing water through their skins and usually lose water in the same way when desiccated, the histology of frog skin is of interest. Elias and Shapiro (1957) in an excellent paper on the skin of some frogs and toads divide the skin into three major layers, beginning from the
outside, as follows:

1. The epidermis which may or may not be keratinized
2. The stratum spongiosum
3. The stratum compactum

These three layers are further broken down as follows:

1. The epidermis has the three following cell layers:
   A. Outermost layer or stratum corneum which is usually, but not always keratinized.
   B. The stratum granulosum which is always in contact with the stratum corneum, but which may also reach down to the basal boundary of the epidermis giving a mixed appearance.
   C. The stratum germinativum in which the actively dividing cells are located.

2. The stratum spongiosum is a loosely packed layer where most of the chromatophores are located.

3. The stratum compactum is a relatively thick layer which contains considerable connective tissue as well as the skin glands and may account for half the total thickness of the skin.

Spearman (1958) points out that the keratinized layer which is almost universally present in the epidermis of the adult amphibians has not yet been demonstrated in the epidermis of larval forms. He
has further shown that while the keratinized layer is not waterproof it can serve as a framework for phospholipids and fats which are "waterproof" and this may account for some of the increased resistance to desiccation exhibited by adult animals both in the terrestrial and aquatic habitats. This keratinized layer is sloughed frequently and the sloughing has been shown to be under the control of the pituitary gland. This keratinized layer is about 5 \mu thick and may be as thick as 25 \mu in the warts of some toads. Lee and Mercer (1967) have shown that this mechanism is of considerable significance in desert forms that burrow into the earth and aestivate to escape long dry periods. In these animals the keratinized stratum corneum loosens and forms a tough waterproof transparent layer that eventually covers the entire animal with the exception of the external nares and thus effectively protects the animal from desiccation.

Tuft (1962) looked at the eggs of *Xenopus laevis* and found that as in the case in most amphibian eggs there are three jelly coats and two membranes around each egg. Beginning at the outside of the egg the coats are as follows:

1. A sticky outer jelly coat that causes the eggs to stick together and to adhere to aquatic vegetation, rocks, or debris that may be in the breeding pool.

2. A stiff middle jelly coat that invests each egg.

3. An inner softer jelly coat.

4. The vitelline membrane that closely invests the egg but allows
a small amount of space for the perivitelline fluid and hence for the rotation of the fertilized eggs.

5. The surface of the egg itself.

Freeman (1968) working with the same eggs found the three jelly coats described by Tuft (1962) and also found that they give slightly different histochemical reactions but that they were all composed of proteins and polysaccharides. He measured and characterized the layers as follows:

1. The outer layer was 0.1 mm thick and very sticky.
2. The middle layer was 0.02 mm thick and was tough and membranous.
3. The inner layer was 0.2 mm wide and gelatinous.

While the foregoing work pertains specifically to the eggs of one animal it is typical of the eggs of most amphibia with minor modifications that allow for adaptations to the special situation in which each animal finds itself.

Salthe (1963) working with the eggs of 41 species of frogs, and 33 species of salamanders found that water, oxygen, CO₂, and NH₃ pass freely through the jelly layers and membranes surrounding the eggs thus allowing for the metabolic needs of the developing embryo. He further noted that in most cases there is a general softening of the jelly layers, and especially the tough middle layer, as the embryo develops. This makes room for the developing embryo and for the
physical movement of the embryo as hatching approaches. When the time comes for the embryo to escape, a hatching gland on its head elucidates enzymes that dissolve a path through the jelly coats. Haglund and Loeffler (1969) showed that while there was a fairly rapid movement of biologically important molecules across the jelly coats of the eggs there also was a measurable resistance to such a flow indicating that these coatings gave at least a small measure of osmotic protection to the developing embryos. Holtfreter (1943) working with *Rana pipiens* and *Triturus torosus* found that various salts will pass rather freely through the jelly layers and he noted that if the concentration of these salts exceeded 3.8% there was a marked softening of the external epithelium of the embryo with a resulting physical distortion similar to that seen if the animals were in an alkaline, or a Ca
sup+sup+ free salt solution.

As noted earlier in this discussion *Hyla regilla* occurs in a wide range of habitats in the State of Oregon. These include the very edge of the sea, the mild Willamette Valley, the Oregon Cascade Range, and the mountain ranges and sagebrush desert of Eastern Oregon. A species with a habitat this diverse immediately becomes interesting from the point of view of limits of tolerance, and special adaptations to local situations. Obviously this study cannot exhaust all the areas of adaptation to specific environments or all the limits of plasticity of the species in meeting a range of
conditions, and hence it will be limited to the following considerations:

1. Is there a difference in salinity tolerance between the coastal, Willamette Valley, Cascade Mountain, and Eastern Oregon populations of *Hyla regilla*?

2. How widespread are any differences that may occur?

3. If there is a difference in tolerance between the populations, what is the mechanism?

4. How do adults, larvae, and eggs compare with respect to salinity tolerance?

5. What is the effect of salinity on eggs and embryos?

6. Is there a physiological justification, based on salinity tolerance, for the establishment of the four subspecies of *Hyla regilla* that occur in Oregon?
MATERIALS AND METHODS

Adult, larval and embryonic stages of *Hyla regilla* were collected and tested in this study. Most of the adults were taken just after dark on rainy evenings along country roads. The one exception was a population sampled near Stinkwater Pass about 80 kms. east of Burns, Oregon, where animals were collected just as they were emerging from hibernation. The frogs were found in shallow burrows dug under the sagebrush by small mammals and in the skeleton of a cow and the debris that had accumulated around it. In no case were frogs from this sampling found more than 4 m. from a permanent backwater pool of the Malheur River. It is of interest that all 50 animals collected at this site were males.

Eggs and tadpoles were taken with a small dipnet from pools in which they occurred. Due to some difficulties encountered in collecting adults over such a wide sampling area, adults were taken in only a few locations. Eggs and tadpoles were collected at all the sampling sites and were grown to the desired stage in the laboratory.

Collections were made within Oregon in the areas designated by Jameson, Mackey and Richmond (1966) as the ranges of the four subspecies of *Hyla regilla* occurring in Oregon. The collecting sites for the animals used in this study are as follows:
Hyla regilla pacifica

A. Animals collected along the shores of the Pacific Ocean.

1. Yachats
   In a depression in the rocks 15 meters from mean high tide, 800 meters S. of Yachats in Sec. 27, T14S, R12W, Lincoln Co.

2. Neptune State Park
   In a seep at the base of a bluff within 15 meters of mean high tide, about eight kms. S. of Yachats in Sec. 10, T15S, R12W, Lane Co.

B. Animals collected about 32 kms. from the head of tidewater.

1. Alsea
   In a pool in a flooded meadow 3 kms. W. of Alsea, in Sec. 9, T14S, R8W, Benton Co.

2. Summit
   In a roadside ditch 5 kms. W. of Summit, in Sec. 30, T10S, R8W, Lincoln Co.

3. Salmon Creek
   In a seep along Salmon Creek which is a tributary of the Yaquina River, about 8 kms. S. E. of Eddyville, in Sec. 23, T11S, R9W, Lincoln Co.
Hyla regilla regilla

A. Animals collected in the Willamette Valley.
   
   1. Orleans Road
      
      On and along Orleans Road, 8 kms. E. of Corvallis,
      in Sec. 9, T15S, R4W, Linn Co.

Hyla regilla cascadae

A. Animals collected in the Oregon Cascades.
   
   1. Three Creeks Lake
      
      In a shallow marsh on the north end of Three Creeks
      Lake, 26 kms. S. of Sisters, in Sec. 14, T17S,
      R9W, Deschutes Co.
   
   2. Summit Spring
      
      In Summit Spring in the ponderosa pine region E. of
      the crest of the Oregon Cascades in Sec. 17, T13S,
      R10E, Jefferson Co.

Hyla regilla palouse

A. Animals collected in Eastern Oregon.
   
   1. Walla Walla River
      
      In a small pool in the canyon of the South Fork of the
      Walla Walla River 16 kms. S. E. of Milton-Freewater,
      in Sec. 4, T4N, R37E, Umatilla Co.
   
   2. Ukiah
      
      In a roadside ditch along Camas Creek in the
headwaters of the Middle Fork of the John Day River
about 3 kms. N.W. of Ukiah in T5S, R31E, Umatilla Co.

3. Stinkwater Pass

In a backwater of the Malheur River along U. S. Route 20 just E. of Stinkwater Pass, about 80 kms. E. of Burns in T20S, R36E, Harney Co.

(No section designations were given for the last two sites since the areas in which they lie have not been surveyed according to the latest U.S.G.S. quadrangle maps available).

All experiments were conducted in a cold laboratory maintained at a constant temperature of 17°C. The adult animals were held in ventilated jars with a small amount of water in the bottom to provide hydration. The experiments involving adult animals were conducted three days after capture to allow the frogs to empty their digestive tracts and come to equilibrium with their surroundings.

Egg masses from which normal animals were desired were divided into small clumps and placed in shallow pans containing 10% Holtfreter's solution. At first the hatchlings were transferred to filtered pond water, but experience showed that deionized tap water was just as effective in producing normal animals and most of the young were reared in this medium. The young tadpoles were fed...
boiled lettuce which they ate readily. Perhaps the addition of the lettuce leaves to the deionized tap water provided the necessary mineral content to satisfy the osmotic and metabolic requirements of the developing tadpoles. In any case no differences in salinity tolerances were seen in tadpoles collected as eggs and reared in deionized tap water and tadpoles collected in the field from the same pools from which the eggs had been collected. Chemical analysis of waters in which frogs were breeding showed surprisingly low salt concentrations with the exception of the pools in the spray zone along the ocean. The pools being fed by snowmelt in central and eastern Oregon gave especially low values. These values are in agreement with data published by the United States Geological Survey regarding the mineral content of most of the major streams in Oregon. This survey indicates that even in the so-called alkali regions in central and eastern Oregon, aside from sinks and alkali lakes, the water carries a very small salt load. Water Resources Data for Oregon, Part 2, Water Quality Records for the Water Year 1966.

Rose (1960) has shown that if tadpoles are crowded, the larger animals elaborate a substance into the water that effectively retards the growth of the smaller animals. To avoid this problem, no more than 15 animals were kept in any one of the rearing pans, and the water was changed twice a week or more often if it was indicated. All tadpoles, whether collected in the field or reared in the
laboratory, were tested at stage 30 (Gosner, 1960), and all animals entered the testing baths with full digestive tracts.

Eggs that were to be tested in graded salt concentrations were cut into clumps containing three to four individual eggs and placed in the appropriate bath. The control was 10% Holtfreter's solution. The eggs selected for these tests were in the early stages of cleavage, usually not beyond eight or 16 blastomeres. The eggs in all baths were allowed to develop until hatching, or in the case of the eggs in the higher concentrations, until development stopped. The animals from this series of experiments were discarded as soon as they were hatched.

Some of the tadpoles were obtained by artificial ovulation and fertilization as described by Rugh (1934). It was found that ovulation could be induced in *Hyla regilla* by implanting female *Rana pipiens* pituitary glands intraperitoneally, or injecting chorionic gonadotropin. Once the female had ovulated, a pair of testes were macerated in a small amount of 10% Holtfreter's solution and the eggs were stripped manually from the female into the sperm solution. In most cases there was nearly 100% fertilization, and the resultant tadpoles were in all cases comparable to those collected in the field.

All animals, except developing embryos, were stressed for a period of 108 hours in a graded series of seawaters. In all cases the dilutions were made from filtered ultraviolet irradiated seawater
of known salinity obtained from the Oregon State University Marine Science Center at Newport, Oregon. Seawater was used in preference to NaCl solutions because it contains a variety of salts in addition to NaCl. It thus would subject the test animals to a stress more nearly approximating that encountered in the field than would a test solution containing only one salt.

After several trials to determine the limits of tolerance of the animals, the following ranges of salinities were selected for the various age classes of frogs:

- **Eggs**: 1.0% to 5.0%
- **Tadpoles**: 5.0% to 11.0%
- **Adults**: 5.0% to 11.0%

In all cases the concentration was increased in increments of 0.5%. In experiments conducted on tadpoles or adult animals six animals were placed in each bath and each concentration was replicated three times making a total of 18 animals tested per concentration per collecting site. When an LD50 was achieved the animals were judged sensitive to the particular salt concentration. The animals were observed at 12 hour intervals and any animal not responding to a touch stimulus was considered dead. Those animals that survived at the termination of an experiment were discarded so that no animal was stressed more than once. In the case of the eggs eight to 12 eggs were tested per salt bath, and these trials were also
replicated three times. Measurements were made of the diameter of the yolk plug, and the diameter of the space enclosed by the vitelline membrane was measured at the neurula, tail bud, gill circulation, and hatching stages. In addition observations were made of the general development of the embryo.

It was observed that, even in tests run on animals from salt sensitive areas, there were always a few animals that survived the highest levels of salt in the test solutions. This suggested that there was at least a measure of plasticity, with respect to salinity tolerance, in the gene pool.
RESULTS

Three life stages of frogs, including larvae, adults, and eggs were collected and subjected to differential salinities. The results will be reported in the same order.

Larvae from the *Hyla regilla pacifica* area were collected directly on the coast and approximately 32 kms. from the head of tidewater. Frogs from the other collecting areas in Oregon were taken in random locations. The salinities at which LD50's were achieved are shown in Table 1.

Table 1. Salinities at which LD50's were achieved in animals from the various sampling areas.

<table>
<thead>
<tr>
<th>Subspecies area</th>
<th>Site</th>
<th>LD50</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyla regilla pacifica</em></td>
<td>Yachats</td>
<td>9.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neptune</td>
<td>9.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summit</td>
<td>8.8%</td>
<td>8.8% to</td>
</tr>
<tr>
<td></td>
<td>Alsea</td>
<td>10.2%</td>
<td>10.2%</td>
</tr>
<tr>
<td></td>
<td>Salmon Creek</td>
<td>9.2%</td>
<td></td>
</tr>
<tr>
<td><em>Hyla regilla regilla</em></td>
<td>Orleans Road</td>
<td>7.2%</td>
<td></td>
</tr>
<tr>
<td><em>Hyla regilla cascadae</em></td>
<td>Three Creek Lake</td>
<td>7.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summit Spring</td>
<td>8.2%</td>
<td>7.2% to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.0%</td>
</tr>
<tr>
<td><em>Hyla regilla palouse</em></td>
<td>Walla Walla River</td>
<td>7.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Camas Creek</td>
<td>7.3%</td>
<td></td>
</tr>
</tbody>
</table>
It should be noted that larvae from the *Hyla regilla pacifica* area, whether taken directly on the coast or 32 kms. from the head of tidewater gave LD50's at much higher salinities than the larvae taken from the three other areas.

Adult animals were taken in both the *Hyla regilla regilla* and the *Hyla regilla palouse* areas, and stressed in graded seawaters. The animals from both areas gave LD50's at 9.5‰.

Eggs and very early embryos from the *Hyla regilla pacifica* and *Hyla regilla regilla* areas were also stressed in graded seawaters and followed until the animals in the control solutions hatched. The animals in the higher concentrations tended to hatch more slowly than those in lower concentrations and the LD50's are based on the viability of all embryos when the most mature began to hatch. At this stage animals from the *Hyla regilla pacifica* area gave LD50's at 5.0‰, while those from the *Hyla regilla regilla* area gave LD50's at 3.3‰.

In addition, the developing embryos in the higher salt concentrations showed several anomalies not seen in the control animals. The first difference noted was in the diameter of the yolk plug. Eggs taken from a single mating as well as those taken at random in the field invariably showed a direct relationship between yolk plug diameter and the salinity of the ambient medium. This relationship is shown in Graph I. In addition it was also noted that while eggs
Graph I. Comparison of yolk plug diameter as function of salinity. Coast frogs and Valley frogs.
from salt tolerant frogs had larger yolk plugs than those from sensitive frogs. The increment of change in response to elevated salinities was about twice as great in the eggs from the sensitive frogs. This relationship is also shown in Graph I.

All animals in the higher salt concentrations showed marked deformities in the area of the blastopore. In some cases closure was greatly delayed or deformed and at the highest salt levels the blastopores failed to close. Neurulation was also affected by the salt concentration with the neural tubes failing to close in some animals at 4.5%, and most of the animals at 5.0% in the area immediately posterior to the hindbrain. In a few instances the neural plate broke along its midline and allowed some of the yolk material to protrude through. The animals in the higher salt concentrations tended to be misshapen and abnormally developed when compared with the controls.

A further consequence of high salt concentrations in the ambient medium was the constriction of the vitelline membrane, that closely invests the embryo. Graph II indicates that animals from salt tolerant areas have a greater resistance to desiccation and consequent constriction of the membrane at all salinities than animals from salt sensitive areas. The result of this inverse relationship between membrane diameter and salt concentration exerted its greatest observed effect at the tailbud stage of development, by forcing the animals' tails to grow at right angles to their normal plane of
Graph II. Comparison of vitelline membrane diameter as a function of salinity. Coast frogs and Valley frogs.
development. This condition, which I have called "bent tail," persists after hatching and markedly impairs the animal's ability to move about.

A final observation relating to the tolerance of the animals is that in all cases eggs proved less tolerant than larvae, and larvae proved less tolerant than adults. Furthermore if animals in one stage were collected in a salt tolerant area the other two stages showed greater tolerance than animals in similar stages collected in salt sensitive areas. The same reasoning applies in the reverse situation. Table 2, while not complete in all cases, gives evidence of this trend.

The sodium content of the waters in which the frogs were collected was determined and is given in Table 3.
Table 2. Summaries of the salinities at which LD50’s were achieved in the three life stages of frogs collected in the four subspecies areas in Oregon.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Hyla regilla pacifica</th>
<th>Hyla regilla regilla</th>
<th>Hyla regilla cascadae</th>
<th>Hyla regilla palouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>---</td>
<td>9.5%</td>
<td>---</td>
<td>9.5%</td>
</tr>
<tr>
<td>Larvae</td>
<td>9.5%</td>
<td>7.2%</td>
<td>7.7%</td>
<td>7.5%</td>
</tr>
<tr>
<td>Eggs</td>
<td>5.0%</td>
<td>3.5%</td>
<td>3.5%</td>
<td>---</td>
</tr>
</tbody>
</table>
Table 3. Sodium content of the waters from which frogs were collected.

<table>
<thead>
<tr>
<th>Subspecies area</th>
<th>Sodium content</th>
<th>Sample taken on the coast</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyla regilla pacifica</em></td>
<td>1000 ppm.</td>
<td></td>
</tr>
<tr>
<td><em>Hyla regilla regilla</em></td>
<td>80 ppm.</td>
<td></td>
</tr>
<tr>
<td><em>Hyla regilla cascadae</em></td>
<td>80-280 ppm.</td>
<td></td>
</tr>
<tr>
<td><em>Hyla regilla palouse</em></td>
<td>20-80 ppm.</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The most interesting observation made in this study is the consistent elevation of salinity tolerance exhibited by all stages of animals collected from the *Hyla regilla pacifica* area when compared with animals from the other subspecies areas. These observations are all the more interesting when one notes that this increased salinity tolerance is uniform throughout the area inhabited by this subspecies and not localized on the coast or in the estuarine habitats of the coastal streams.

Several workers (Hardy, 1943; Spurway, 1943; Taylor, 1943; Ely, 1944; Peterson, Garrett and Lantry, 1952; Ruibal, 1959 and 1961; Gordon, Schmidt-Nielsen and Kelley, 1961; Gordon, 1962 and Ackrill, Hornby and Thomas, 1969) have shown that amphibians have adapted to a wide range of salinities. These run from 2‰ to 3‰ to an extreme value of 39‰ or 120% seawater in *Rana cancrivora* which inhabits mangrove swamps facing the open ocean.

Gordon, Schmidt-Nielsen and Kelley (1961) suggest that most adult amphibians can withstand salinities ranging from 9‰ to 11‰ depending on the species. While no adults were tested from the *Hyla regilla pacifica* area, those from the *Hyla regilla regilla* and *Hyla regilla palouse* areas gave LD50's at 9.5‰ and the larvae from the *Hyla regilla pacifica* areas gave a mean LD50 at 9.5‰. Ruibal (1959) has shown that in *Rana pipiens* there is a progressive tolerance for
greater salt concentrations as one goes from eggs to adults which is in agreement with the data from the *Hyla* subspecies areas where the data are complete. The foregoing considerations leave little doubt that adult *Hyla regilla pacifica* can survive considerably more than 9.5% salt in their ambient medium. Gordon (1962) working with *Bufo viridis* found a range in tolerance of 19% to 23% depending on the area from which the animals were taken. Ruibal (1959) found that a population of *Rana pipiens* living in San Felipe Creek which empties into Southern California's Salton Sea could survive in the laboratory for three months in a salt concentration of 6% but that they would die in three hours if the concentration was raised to 13%. Gordon, Schmidt-Nielsen and Kelley (1961) have shown that adult *Rana cancrivora* live in water with a salt concentration of 28% and can tolerate 32% for shorter periods of time. They have also shown that the larvae of this species can survive in 39% salt concentration. This is the only known exception to the rule that adult amphibians are more tolerant to salt stress than larvae, and the same authors present evidence that these larvae excrete urea rather than ammonia which is also a unique situation and may help to explain the unusual tolerance of these larvae. This is probably a special adaptation to a saline environment, but it does indicate that within a single genus of frogs there is considerable plasticity in the gene pool with respect to salinity tolerance.
It can be seen that the salinity tolerance values for the various subspecies of *Hyla regilla* occurring in Oregon fall within the limits found in other amphibians for which data are available, and the differences in tolerance found in *Hyla regilla* are also within the limits found in other amphibians. During the course of these experiments it was noted that there were always a few larval animals that were able to survive for at least 108 hours in the highest salinities to which the test animals were subjected indicating that there was at least a measure of plasticity in the *Hyla* gene pool. It is logical to postulate that frogs inhabiting the coast and estuaries have, over a period of time, been selected for salinity tolerance and that these tolerant genes have slowly worked their way up the coastal streams and become dominant in the area west of the crest of the Oregon Coast Range. Since these mountains do not form an impassable barrier to *Hyla*, they either represent the extent to which the salt tolerant genes have penetrated inland, and from which they will presumably keep moving inland, or the point at which these genes no longer confer an adaptive advantage and are beginning to be selected against. One then comes to the conclusion that the animals in the three other subspecies areas haven't been subjected to a salt stress with the result that the genes which confer salinity tolerance haven't been selected for and thus the animals from these areas give lower LD50's than the coastal animals even though there is evidence that
some salt tolerant genes exist in a sensitive population.

Many workers have suggested a variety of ways in which the genes could express themselves to bring about the observed tolerance to salinity. These include facilitating rehydration (Packer, 1963; McClanahan, 1964; Dole, 1967; Lee, 1968; Lasiweski and Bartholomew, 1969), toleration of elevated electrolytes (Ray, 1958; Mayhew, 1965; Schmid, 1965; Bentley, Bretz and Schmidt-Nielsen, 1967; and Clausen, 1969), the regulation of fluid loss through the skin in response to endocrines (Adolph, 1932; Ewer, 1950, 1952a, b; Thorson, 1955; and Jasinski and Gorbman, 1967), the retention of large amounts of bladder water (Ruibal, 1962; Shoemaker, 1964; Lee, 1968; and Clausen, 1969) and the formation of nitrogen-bearing excretory products of reduced toxicity that can be stored temporarily in the animal's body to help maintain its osmotic integrity (Munro, 1953; Balinsky, Cragg and Baldwin, 1961; and Cragg, Balinsky and Baldwin, 1961).

Ruibal (1969), working with Rana pipiens, noted that the diameter of the yolk plugs at the end of gastrulation was directly proportional to the salt concentration of the ambient medium. Ruibal always got normal development below 3.8‰ and almost 100% mortality at 5.0‰ or above. He further noted that developing embryos could be placed in a graded series of salinities, on the basis of their yolk plug diameters alone.

Hyla regilla showed the same relationship except that embryos
of *Hyla regilla pacifica* showed LD50's at higher salt concentrations than embryos of the three other subspecies tested in this study. It was also noted that embryos of *Hyla regilla pacifica* had larger yolk plugs than embryos of the other subspecies taken at the same stage of development. In addition the increment of yolk plug enlargement in response to elevated salt levels was about twice as great in salt sensitive animals as it was in salt tolerant animals. Apparently the tolerant animals have achieved an optimal yolk plug diameter that represents the upper limit of that which can develop normally and yet accommodate to salt stress. By contrast sensitive animals haven't achieved this balance and are incapable of maintaining a yolk plug diameter that will allow for a normal closure of the blastopore and the normal development of the related structures.

Holtfreter (1943) showed that the salt concentration of the internal milieu of an amphibian embryo at the neurula stage is about 3.8% and that if the salt level of the ambient medium is raised above this point there is a tendency for the embryo coat to soften in about the same manner as it will in response to slightly alkaline and calcium free solutions. The *Hyla* embryos tested in the higher salt solutions responded in a similar fashion, with neurulation beginning to fail at 4.5%, and failing in most cases at 5.0% in sensitive frogs and at 5.5% and 6.0% in tolerant frogs.

Tuft (1962), Salthe (1963) and Freeman (1968) have all
described the amphibian egg as being surrounded by three distinct jelly coats formed from slightly different mucopolysaccharides with slightly different physical properties. Together these jelly coats serve to protect the integrity of the developing egg. Salthe (1963) showed that there is an exchange of water, oxygen, CO₂ and NH₃, among other things, across the jelly coats as the embryo develops. Haglund and Loeffler (1969) showed that while there is a good exchange of these elements across the jelly coats, there is also a measureable resistance to this flow. Apparently this mechanism is also implicated in the adaptation of Hyla regilla to elevated salt levels since the vitelline membrane of eggs from salt sensitive frogs showed a much more pronounced reaction to high salt concentrations in the ambient medium than do those from salt tolerant frogs. This extreme compression of the vitelline membrane on the embryo exhibited by eggs from salt sensitive frogs in response to an osmotic gradient results in deformed larvae that will be highly selected against. Evidently the resistance to membrane transport shown by Haglund and Loeffler (1969) has been selected for in the salt tolerant animals found west of the Oregon Coast Range with the attendant sparing effect on the developing embryos.

In summary it seems that the animals in the Hyla regilla pacifica area have gone through a process of selection for a rather large number of characteristics that make possible the occupation
of a more saline habitat than is possible for the subspecies found in the other subspecies areas in Oregon. This selection provides a physiological justification, based on elevated salinity tolerance, for the establishment of the subspecies *Hyla regilla pacifica* by Jameson, Mackey and Richmond (1966). The three other subspecies occurring in Oregon can't be separated on the basis of salinity tolerance, but future work may reveal other physiological differences between these subspecies.
BIBLIOGRAPHY


