

Behavior, Morphology, and Ecology of Rough-skinned Newts (*Taricha granulosa*) in Dynamically Saline Environments.

By
Sophia K. Espinoza

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Dr. Gareth R. Hopkins
Thesis Advisor

Dr. Gavin Keulks
Honors Program Director

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Abstract

Aquatic ecosystems can range from freshwater to saline depending on their proximity to natural (e.g., oceanic) or anthropogenic (e.g., road salts) sources of salt. This variation in salinity excludes many osmotically-sensitive freshwater organisms from brackish habitats. Due to their permeable skin and egg cases, amphibians have long been known as highly intolerant of salt water. Studies have highlighted the detrimental physiological effects that heightened salinity causes across all life stages. Therefore, most amphibians should inhabit exclusively freshwater environments. However recent evidence documents amphibians around the world breeding in habitats that differ greatly in salinity.

In coastal environments, salinity of otherwise freshwater habitats can fluctuate across both space and time with tides and storm events, creating a complex matrix of fresh and brackish water. How amphibians navigate this complexity is not well understood. This thesis aimed to document the spatial and temporal variation of salinity using a tidally-influenced system on the Oregon coast, and record Rough-skinned newt (*Taricha granulosa*) distribution, behavior, and morphology at this site in comparison to a constantly freshwater system. Two sites in western Oregon (coastal/saline and inland/freshwater) were selected for their water dynamics and abundance of *T. granulosa*, then surveyed for salinity variation and newt distribution. At the coastal site, these surveys were conducted at intervals

congruent with the major fluctuations of the 24 hour tidal cycle. Additionally, two salinity loggers were placed at the coastal location to measure salinity variance over a period of four months (June-September). At each site, newts were collected and returned to an animal housing facility, where they would spend 24 hours in tanks with water of varying salinity levels. The distribution of newts was recorded to examine any behavioral responses to changing salinity (preference, avoidance, breeding, ovipositing) across microhabitats, and morphology of newts were measured to determine any possible growth tradeoffs associated with saline environments.

The coastal site experienced large fluctuations throughout a 24 hour tidal cycle in the stream sections (0.06 - 25.28 ppt), whereas the connected lake experienced high salinity spikes, then constant low salinity lasting over seven days. Large differences in salinity based on depth were also recorded, with measurements ranging from 0.42 ppt to 12.98 ppt in as little as 50 centimeters from water surface to bed. Coastal newts showed significant variation in spatial and temporal distribution during this cycle. Newts were recorded inhabiting parts of the coastal habitat in salinities ranging from 0.0 to 25.28 ppt (sea-water ≥ 32 ppt), beyond what is currently recognized as the experimental salinity tolerance of this species. Morphometric data of newts revealed that coastal newts were significantly smaller compared to the inland newt population, indicating a potential cost to living in this variable habitat. Laboratory results revealed a trend of both inland and coastal

newts preferring a small amount of salinity (0.5 ppt or less), but inland newts preferring freshwater in treatments of higher salinity (6.00 ppt or more), whereas coastal newts did not exhibit a strong preference between the higher levels of salinity water treatments. As anthropogenic influences increase salt quantities in aquatic environments, better understanding the spatio-temporal variability of salinity in these habitats will be crucial to accurately predict the impacts of salinization on amphibians and other osmotically-sensitive freshwater organisms.

Introduction

Amphibians have highly permeable skin, which is used for cutaneous respiration and osmoregulation. Their sensitive skin will react to subtle changes in the environment, such as chemical, temperature, or UV factors. This makes them excellent indicators of change in the ecosystems that they inhabit. A common area of change that many amphibians are facing is an increase in salinity in aquatic systems. As climate change continues to alter the landscape of aqueous habitats through weather patterns such as storm surges, rising sea levels, and anthropogenic sources, long-term salinity levels and salinity spikes are becoming increasingly intense.

Amphibians who are regularly exposed to saline waters during their development as tadpoles often experience adverse effects, such as higher mortality rate during development and negatively impacted tolerance to heat and physical performance as adults (Chuang, 2022). Additionally, Amphibian populations can experience detrimental effects to their routine breeding behaviors due to salinity encroachment into viable breeding and reproductive sites (Yiming 2013). The need to understand amphibian response and adaptation to osmotically stressful environments is more important than ever. Though there have been many documented observations of amphibians in saline environments, there has been a

lack of investigation into their behavior and life history in relation to the environment's salinity levels. A failure to efficiently categorize these occurrences has led to ill-defined relationships with these saline environments (Hopkins and Brodie, 2015).

There is little standardized record of the duration and use of the saline environment by amphibians, how salinity in these environments changes over time, and the health of the animals utilizing those habitats. The absence of detail results in a paucity of knowledge on how amphibians interact with salinity during their life history and how the varying salinity can affect a species' migration patterns, reproductive behaviors and success, and species abundance. This thesis aims to detail the short term and long term changes in salinity in an amphibian habitat, documenting the morphological and behavioral response of an amphibian species in this variable environment.

Literature Review

This synthesis aims to address key findings from prior research and emphasize the need for more investigation into the effects of varying salinity levels on amphibians. I will examine salinity as an environmental factor in amphibian habitats; breeding/oviposition site selection in response to environmental features (abiotic and biotic); and species-specific responses to environmental and experimental salinity. Amphibians are an excellent group to study for this topic due to their sensitivity to osmotically stressful environments and the fact that a large majority of amphibians require water to rear their young, from oviposition to larval development.

Previously, there has been research that has observed the existence of amphibians in saline environments, both detailed reports and sparse field observations (Hopkins and Brodie 2015). Though this has been documented more rigorously in the last decade, missing information remains. Understanding the habitats and ecosystems that amphibians face on a daily basis is crucial to comprehending behavioral responses and creating effective conservation strategies and management plans.

Methods of Review

The literature review portion of this study was completed using scholarly digital inquiries on the Google Scholar online engine, with key words “amphibians”, “salinity”, “tolerance”, “dynamically saline”, and “osmoregulation”, used to query results. The query was not sorted by age, but rather the set sorting method used by Google Scholar to organize literature based on key word relevancy. The first 100 results were examined for accuracy and relevance to the project. Review results were then categorized into bins based on the relevant main topics of the paper and defined in the following categories:

1. Salinity as an Environmental Factor in Amphibian Habitats
2. Species-Specific Responses to Salinity
3. Oviposition and Site Selection in Response to Biotic and Abiotic
Environmental Features (Salinity Focus)

Salinity as an Environmental Factor in Amphibian Habitats

This category encapsulates any and all reports that provide data on salinity in amphibian habitats. This includes the different sources of salinity in habitats and their varying levels, from both natural and anthropogenic causes. This also includes any information in the literature that discusses possible salinity and other (a)biotic factors (water levels, depth, competition) in the environments they inhabit. In addition, studies that have recorded the physiological impacts that salinity exposure has on amphibians (short-term and long-term exposure, permanent exposure, recovery) have been included.

Species-Specific Responses to Environmental and Experimental Salinity

This grouping of data includes current information regarding freshwater-dependent species of amphibians, comparing them directly to saline-tolerant species in laboratory-based experiments and observational studies. Any documentation of amphibians in brackish or saline environments are included in this category. In addition, literature discussing the life history and evolutionary adaptations that have contributed to saline/brackish tolerance are present in this grouping.

Breeding/Oviposition Site Selection in Response to Environmental Features

(Abiotic and Biotic)

Findings related to breeding and oviposition behavioral changes in response to a varying environment are organized within this group. This also includes studies that have recorded data regarding site fidelity across amphibian habitats and detection for characteristics of sites. Lastly, this grouping will incorporate discussions of the trade-offs between habitat competition, predation risks and avoidances, and how environmental tolerance influences breeding behaviors.

Results of Review

Salinity as an Environmental Factor in Amphibian Habitats

Varying Environmental Salinity in Coastal Habitats

Coastal water bodies are prone to varying salinity levels due to tidal surges, sea spray, and ocean storms, that all cause salinization of the water (Lorrain-Soligon et al., 2021). Some habitats return to their original salinity levels, such as after a usual tidal pattern. Others become highly saline after large-scale ocean storms that sweep the coast. Long-term salinity logging off the northern coast of France in *Bufo spinosus* habitat has indicated that some sites were not able to return to their normal salinity levels after large coastal storm events (Lorrain-Soligon et al., 2021). Due to the now highly saline waters, amphibian richness has not returned to

the levels present pre-salinization (Lorrain-Soligon et al., 2021). As freshwater continues to become more saline, this osmotic stress could pose large threats to amphibian populations if they are unable to adapt (Hopkins et al, 2016).

Physiological Impacts of Salinity on Amphibians

Salinity has a nuanced effect on osmotically sensitive amphibians. Though species may be closely related, even sharing a genus, many exhibit differing reactions to saline environments. This can be seen in the *Hyla* genus of frogs, where inland *Hyla chrysocelis* and *Hyla squirella* in the eastern United States exhibited a higher survival rate in saline waters than the coastal *Hyla cinerea* (Albecker and McCoy, 2023). Despite *Hyla cinerea* being the most coastally prevalent of the three species, negative impacts of salinity exposure have been documented (Albecker and McCoy, 2019). This suggests that there may be a physiological disposition for salinity tolerance in more amphibians than those recorded in presently saline environments.

Despite this possibility, variable exposure poses a varying level of risk for different species. *Hyla* tadpoles introduced to salinity during their development have a faster development rate and smaller final size than inland species, speculated as an escape response to osmotically stressful environments (Albecker and McCoy, 2023). This is seen in *Bufo spinosus* as well. *Bufo spinosus* tadpoles exposed to medium- and long-term saline environments demonstrated higher

mortality rates, decreased growth, and lower activity (Lorrain-Soligon, 2024).

Large and sudden changes in salinity increased responses to predation stimuli, but decreased overall activity and led to detrimental effects within less than one hour of exposure (Lorrain-Soligon, 2024).

In both the *Hyla* and *Bufo* species, introduction to salinity during larval and tadpole life stages did not create a positive impact on tolerance later in life (Albecker and McCoy, 2019, 2023, Lorrain-Soligon, 2024). Additionally, reintroduction to freshwater did not guarantee an elimination of salinity-induced effects. *Bufo spinosus* tadpoles given osmotic relief after two weeks of salinity exposure exhibited the same behaviors as when in their previous environment (Lorrain-Soligon, 2024).

Such physiological changes and effects have been documented in coastal and inland populations of *Buergeria japonica* and *Buergeria buergeri* frogs in Japan (Haramura et al., 2019). *B. japonica*, which inhabits a variety of habitats from mountainous to coastal, was speculated to have a different osmoregulatory system than *B. buergeri*, which lives solely in freshwater. An observation of variation in osmoregulatory hormones ANP (atrial natriuretic peptide) and AVT (arginine vasotocin) revealed substantial differences between adults of both species, with *B. japonica* exhibiting much higher levels of ANP while resting (Haramura et al., 2019). This indicates that *B. japonica* may have an anticipatory mechanism to

preemptively combat any introduction to high levels of salinity, common in their coastal ranges of Taiwan and the Ryukuku archipelago (Haramura et al., 2019). That said, return to freshwater after high salinity exposure showed two of five *B. japonica* dying within 4 hours of the transfer (Uchiyama, 2014).

Much like the *Hyla* and *Bufo* frogs, this data indicates that salinity tolerance, though likely more widespread than previously thought, may be viable for limited times (up to four hours in $\frac{1}{2}$ parts saltwater and up to six hours in $\frac{1}{3}$ parts saltwater), resulting in lasting detrimental effects after the waters' return to stability (Haramura et al., 2019). Long-term exposure to salinity now indicates that there is a timeline or limit to preparatory mechanisms present in species like *B. japonica* and *B. buergeri* but does not address environmental salinity fluctuations caused by tides, sea spray, storm events, and other coastal weather events.

Species-Specific Responses to Salinity

As a result of their physiology, many amphibians respond to salinity exposure in a variety of ways. This has led to occurrences of amphibians in environments that were previously considered too osmotically stressful for permanent inhabitants (Hopkins and Brodie, 2015). The cause of variable distribution within saline environments is hypothesized to be a combination of factors, including physiological attributes and salinity detection capabilities. Anurans such as *Buergeria japonica* reportedly possess preparatory osmoregulation mechanisms, which could combat the salinity variations in the coast they inhabit (Haramura et al., 2019). The possibility of salinity detection is another potential method to avoid salinity avoidance.

In the inconsistently saline waterways, rivers, streams, and bays of the Oregon coast, Pacific tree frogs (*Pseudacris regilla*) and Northern red-legged frogs (*Rana aurora*) reside. The Pacific tree frog has a general salinity tolerance of up to 10 ppt, whereas the Northern red-legged frog has a tolerance of beyond 5 ppt (Hopkins and Brodie 2015, Goddard 2018). During field observations, Pacific tree frogs were recorded in naturally saline water bodies with no avoidance behaviors exhibited (Goddard, 2018). The Northern red-legged frogs, with a higher tolerance in the lab, seemed avoidant of highly saline water bodies and were less abundant here during surveys (Goddard 2018).

Additionally, possible salinity detection has also been recorded in experiments examining the effects of common road de-icers containing NaCl on the behavior of Northern Spring Peepers (*Pseudacris crucifer*) (Lindstrom, 2014). When introduced to saline environments, *P. crucifer* exhibited avoidance behaviors synonymous with detection abilities and preferred lower salinity environments (Lindstrom, 2014). This avoidance response was exhibited a few minutes after exposure to saline water but dulled in ability and response behaviors past the initial detection period, falling short of long-term detection and response (Lindstrom, 2014). This is indicative that a time-constrained salinity detection capability may be present as a result of adaptations to intermittently saline environments. Further salinization of amphibian habitats could contribute to the high decline of populations by eliminating viable breeding habitats. Increased desiccation rates may also contribute to population declines.

Breeding/Oviposition Site Selection in Response to Environmental Features

(Abiotic and Biotic)

Behavioral Responses to Salinity During Breeding

With coastal amphibian habitats becoming increasingly saline, understanding the impact of salinization, through routine and/or chance events, on breeding site selection and oviposition behavior is crucial to conserving species. Many amphibians, like the Spadefoot Toad (*Pelobates cultripes*), exhibit high site fidelity and return to their original breeding grounds year after year (Lorrain-Soligon, 2024). Coastal *P. cultripes* have been documented to have higher annual migration rates than inland populations. The cause of this, however, is still unknown (Lorrain-Soligon, 2023). Energy trade-offs between growth rate and final size (hypothesized as an escape mechanism from osmotically stressful environments) are also speculated to contribute to migration distances and behaviors, altering breeding adults' site decisions (Lorrain-Soligon, 2023).

Salinity has not been thoroughly studied as a breeding site-defining characteristic, though there is data suggesting that some amphibians select their breeding/oviposition sites based on environmental factors. A tree-hole breeding frog known as the Guinea river frog, *Phrynobatrachus guineensis*, has demonstrated perception of site characteristics and altered its behavior accordingly (Rudolf and Rodel, 2005). When monitored, *P. guineensis* had preferences for sites that met their requirements of maximal water depth, sediment deposit, and water

retention (Rudolf and Rodel, 2005). These factors heavily influenced female *P. guineensis* oviposition decisions.

Site selection is also reported to be temperature dependent as well. Boreal toads, *Anaxyrus boreas boreas*, have been documented interacting with water of varying temperatures before and after the introduction of *Batrachochytrium dendrobatidis* (Bd) (Barrile et al., 2021). During observations, *A. boreas boreas* did not exhibit any detection or consideration of waters infected with Bd, so no avoidance was recorded. After the introduction and infection of Bd, however, the toads would seek out water with higher temperatures, associated with a higher body temperature and clearing of the infection (Barrile et al., 2021). This appears to be a selective pressure that has influenced the behavior of the toads, noting that the only three infected toads that did not seek out warmer water died. The application of these site-selective characteristics remains unknown when considering the salinity of breeding/oviposition habitats, though there is data that suggests it may affect breeding adults' habitat choice in the future. Wood Frogs (*Lithobates sylvaticus*), were observed selecting sites that mimic the habitats they were reared in. (Vesgo et al., 2022). *L. sylvaticus* that were reared in higher salinity environments until their maturity were reported to show preference for the same higher saline environments as adults (Vesgo et al., 2022). The inverse pattern was present in the frogs reared in lower salinity environments (Vesgo et al., 2022). In addition, there has been no correlation found between selection for higher salinity

sites and higher rates of desiccation in juvenile-adult populations, indicating that salinity detection abilities may be working in conjunction with life-history preference to shape these habitat distributions.

Working in close partnership with characteristic preferences in breeding habitats is the energy trade-off documented in multiple species (Lorrain-Soligon 2021, Furness 2022). Factors such as local predation, competition, and environmental variation often pose stressors for developing amphibians. *P. cultripipes* observed on the coasts of France have exhibited distributions that display the effects of these factors. Documentation of their reproductive success, abundance, size, sex, and age ratio in relation to the environmental salinity determined that salinity is a strong determining factor of size and sex distribution (Lorrain-Soligon, 2021). Males are smaller and develop faster in brackish water than their freshwater counterparts, due to what is speculated to be a sex-dependent distribution pattern. The data collected shows a strong indication that more favorable breeding conditions rear smaller broods with larger offspring, whereas stressful environments result in larger broods of smaller final developmental size (Furness, 2022). This size reduction/difference can have negative implications for predatory defense and reproductive success. With habitats becoming increasingly stressful, the negative impacts of energy trade-offs are likely to become more prevalent as coastal environments become increasingly saline.

Amphibians' biology makes them highly susceptible to changes in the aquatic habitats in which they live. Varying levels of salinity create larger impacts on these osmotically sensitive organisms and as a result, their morphology and behaviors shift in response.

Our understanding of the role that salinity plays in amphibians' lives is continuing to grow and we are beginning to grasp the impact that it has on the survival of populations in dynamically saline environments. Responses to salinity vary, ranging from phenotypic plasticity, and morphological changes to growth patterns, site selection, breeding behaviors, and life history trajectory. Both natural and anthropogenic induced salinization is causing uncharacteristic changes to formerly freshwater habitats that amphibians use for breeding, oviposition, and rearing. Understanding the implications of rising salinity on these osmotically sensitive species is more important than ever. In-depth data must be collected on the impacts of rising salinization on coastal amphibians in dynamically saline environments to continue creating habitat and species management strategies that will most effectively benefit them.

Thesis Objectives

This thesis aimed to address the gaps in methodological and empirical knowledge in how amphibians tolerate and navigate environments that are under varying levels of change, ranging from predictable cycles, to strong stochastic fluctuations. We focused on the variation of salinity within freshwater and dynamically saline habitats, looking closely at how salinity can vary across the space within a habitat, as well as across time frames ranging from hours to months. Additionally, we wanted to document the morphology and characteristics of amphibians that were living in dynamically saline environments and compare them to that of animals that lived in completely freshwater.

The purpose of this comparison of inland and coastal populations was to consider and evaluate the potential “cost to living” or energetic “trade off” scenario that may be associated with living in an unideal habitat, such as a saline waterbody. We chose to meet these objectives using the common, wide-spread amphibian, the rough-skinned newt (*Taricha granulosa*), which has been previously found in a variety of coastal habitats in Oregon (Hopkins et al 2016; Goddard 2018).

Specifically, we aimed to:

1. Measure the spatial and temporal variation of salinity in a coastally located, dynamically saline habitat over the timespan of hours to months.

2. Document the morphological characteristics of Rough-skinned newts (*Taricha granulosa*) in coastally located, dynamically saline environments and constantly freshwater (inland) environments.
3. Document the behavior of female Rough-skinned newts from fresh and saline habitats when given the experimental choice of water bodies of varying salinity within a 24 hours time frame in the laboratory.

Site Overviews

This study was conducted at two separate locations with separate populations of rough-skinned newts. Both sites, as seen below, were located within the northwestern portion of Oregon, less than 100 miles in proximity from one another (Fig 1.). One site, Muddy Valley Habitat Reserve, was an inland freshwater site with a large population of newts that occur in strong numbers annually. The second site, Reynolds Creek /Placer Lake, was located coastally. These two locations were selected for their representations of varying salinity within amphibian habitats, as well as their high numbers of newts. Both sites were visited and sampled with the permissions of their respective land stewards.

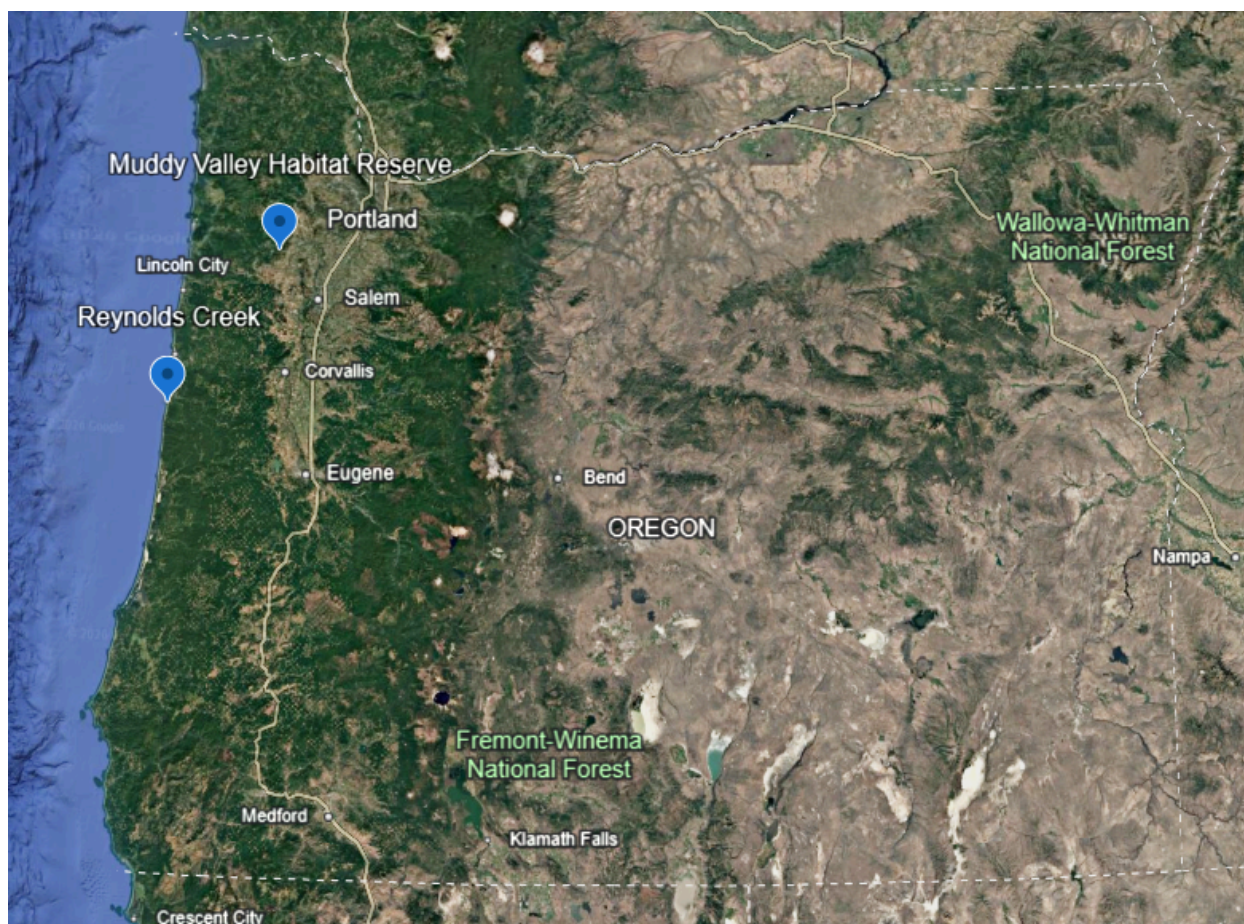


Fig 1. Aerial representation of inland (Muddy Valley) and coastal (Reynolds Creek/Placer Lake) sites. Geographic location of sites represented with blue pin icons. GoogleLandsat / CopernicusData LDEO-Columbia, NSF, NOAAData SIO, NOAA, U.S. Navy, NGA, GEBCO.

Reynolds Creek and Placer Lake, Lincoln CO., Oregon

Located on the Oregon coast in Lincoln County, Reynolds Creek and Placer Lake rest just north of the Tillicum Beach State Park, roughly 3.5 miles north of Yachats, Oregon. The connected lake and creek are stewarded by the United States Forest Service as a part of the Siuslaw National Forest, which spans 991 square miles along the central Oregon coast. The water system observed contains four major aquatic components, all of which are connected (Fig 2). At the easternmost reaches is Placer Lake, fed by two small freshwater streams (Fig 2D). Placer Lake feeds directly into Reynolds Creek, which stretches west towards the ocean. In a short distance, Reynolds Creek is joined by Big Creek (Fig 2B). Together, they flow out into the Pacific Ocean. The entire water system from lake to ocean is roughly 800 meters (0.5 miles) long, as the crow flies (Fig 2).

The water system is heavily influenced by the tides of the ocean, experiencing periods of high swells as well as low water. The wide mouth of the inlet allows for tidal influxes to push back against the flow of Reynolds and Big Creek. Substrate varies along the system, but is composed of primarily porous, sandy substrate, layered with decomposing vegetation and muddy silt further into the lake area. The floor of the water bodies varies incredibly within the small stretch of land it occupies. Wide and shallow channels narrow and deepen as distance from the ocean increases. Mud, driftwood, and vegetation creates pits, false bottoms, and troughs along the creek and lake beds (e.g., Fig 2C). Additionally, Highway 101

rests above the water's path to the ocean, placing it in the path of possible anthropogenic interference/damage.



Fig 2. The Reynolds Creek and Placer Lake waterbody in Lincoln County, Oregon (A). Cyan (B), yellow (C), and magenta (D) dots mark examples of distinct micro characteristics in the water system.

Muddy Valley Habitat Reserve, Yamhill CO., Oregon

Muddy Valley Habitat Reserve is located in Yamhill County, Oregon, Southeast of McMinnville (Fig 3). This 341 acre parcel of land is owned by the Yamhill Soil and Water Conservation District. The land is a part of the Willamette River Basin Memorandum of Agreement Regarding Wildlife Habitat Protection and Enhancement that was signed by the Bonneville Power Administration and the State of Oregon in 2010. This land serves as protected habitat for many of Oregon's native species and ecosystems, such as the Northwestern Pond Turtle (*Actinemys marmorata*) and oak savannah habitat. This protected habitat consists of a system of ponds and oak savannah (Fig 3).

The observed pond, labeled "Pond A", is the northernmost pond, located at the highest elevation out of the many waterbodies (Fig 3B). Pond A is closed off from all other water bodies and is fed by a small freshwater stream to the west. All other water comes from groundwater run-off and precipitation. The pond is even in depth and characteristics across the perimeter, with a small shallow bank at the northern side and western side, where the water source originates. Pond A is surrounded by protected land at a higher elevation than the surrounding developments, preventing agricultural runoff into the habitat. Additionally, low vehicle and personnel traffic on the site reduce the amount of harmful chemicals that may infiltrate the habitat.



Fig 3. (a) Muddy Valley Habitat Reserve and (b) the survey location, Pond A . located in a (c) managed oak savannah ecosystem. GoogleLandsat / CopernicusData LDEO-Columbia, NSF, NOAAData SIO, NOAA, U.S. Navy, NGA, GEBCO.

Study Site Field Methods

Survey Methods

Both Reynolds Creek and Muddy Valley (MVHR) were surveyed in May of 2025 to assess salinity levels, as well as newt presence and morphometrics. Surveys were conducted over the course of one day at MVHR and over two days at Reynolds Creek. Both field sites were surveyed using a perimeter visual encounter survey protocol. During the traverse of the perimeter of each water body, personnel would visually inspect the water for newts. Upon encounter, time and geographic coordinates at that location were recorded using the mobile application, “GPS Data”.

After the coordinates were recorded, personnel would collect up to six newts haphazardly. Newts were collected using dip nets and hand capture. These newts were then sexed, measured by snout-vent-length and total length, weighed, and then released at the same location. Simultaneously, water temperature, pH, and conductivity were recorded using a handheld YSI Pro30 conductivity probe.



Fig 4. Survey protocol for the research sites, including the collection (a), measurements (b), behaviors (e.g., a newt is ovipositing at Reynolds Creek in this photo) (c), and water characteristics documentation (d), performed at both Reynolds Creek and Muddy Valley Habitat Reserve in May and June of 2025.

Surveys were conducted once at MVHR and four separate times at Reynolds Creek. Increased surveys at Reynolds Creek were conducted to account for the four distinct tidal events that take place on the Oregon coast within a 24 hour timeframe. The west coast of the United States experiences a mixed semidiurnal tide which consists of one true high tide and one true low tide, with an additional high and low of lesser magnitude. Each tidal event was only surveyed once, and each perimeter sweep spanned roughly three hours from start to finish, totaling 12 hours of surveying within a 24 hour cycle.

In addition to survey efforts, long term water chemistry data was collected with the implementation of two stationary HOBO Conductivity/Salinity Data Loggers (Part # U24-002-C). These loggers were placed at two separate locations at Reynolds Creek where newts were found (Fig 5); the first of which was at the easternmost side of Placer Lake in a shallow area (>1 meter deep; Fig 5C), with the other place just before the meeting of Reynolds Creek and Big Creek at a further point west where newts were encountered (~1 meter deep; Fig 5B). Loggers were mounted to the base of a T-Post and marked with labeled tape at the top. These two locations were chosen to provide a contrast to one another and provide insight on the many microhabitats in the water system. These loggers were deployed in May (one logger) and June (the other logger) and monitored via routine check-ins. Loggers were programmed to record temperature and conductivity at a 15 minute interval for the duration of their deployment. The

loggers were then extracted from the site in September. No loggers were placed at MVHR due to the lack of variance in salinity across seasons.



Fig 5. Hobo Conductivity Logger location in the Placer Lake and Reynolds Creek water system (a), with Logger A (b) placed in the main channel and Logger B (c) at the eastern most end of the lake. Blue stars represent their geographic location in the waterbody (a) and their unique ID is represented in the adjacent yellow letters A and B.

Collection of Newts from Study Sites

At the conclusion of the surveys, 25 gravid female newts were collected from each respective location for a total of 50 newts. Gravid females were collected throughout the duration of the final survey and placed in a holding bucket. After the end of the survey, 25 females were haphazardly collected from the bucket to avoid selection bias for a specific area of the water system. The surplus newts were re-released into the water less than 500 meters from their original collection location. Collections were performed by the PI, faculty advisor, and trained student volunteers under a collections permit approved by the Oregon Department of Fish and Wildlife.



Fig 6. Final collection of female rough-skinned newts that were haphazardly collected from each location. 25 individuals from each location were selected, with the rest being returned within 500 meters of their original collection location.

Laboratory Methods

All 50 gravid female newts were returned to Western Oregon University under the approval of Institutional Animal Care and Use Committee protocol IACUC-25-16. These newts were held in a dedicated, IACUC approved animal holding facility from their collection date in May of 2025 until September 2025. Upon arrival, each newts was measured for snout-vent-length, total length, weighed, and assigned a unique ID code to monitor each individual (Fig 7).

Housing containers were stored in a Percival temperature-controlled chamber. These chambers were set to a “day” temperature of 14 degrees celsius and a “night” temperature of 13 degrees celsius, which was within the range of temperatures newts experienced in the field in both locations. Lights within the chambers were set on a 1:1 ratio, with 12 hours a day of light and 12 hours a day of dark. This was set to mimic the estimated evenness of the diurnal cycle in Oregon during the months of May/June. Day and Night temperature followed this same pattern. Newts were housed in individual containers with water and a styrofoam perch. Each container was roughly 5” x 11” x 5” and filled $\frac{2}{3}$ full with de-ionized and de-chlorinated water. Unique IDs from processing were marked on the containers to ensure that there was no cross contamination of containers during water changes and feeding (Fig 7).

The water in all containers was changed no less than once a week, often more to remove waste, shedding, and other biomaterials. Additionally, all newts were fed every three days with blood worms and small earthworms to maintain nutritional balance. All water changes and feeding schedules were kept track of manually using an organizational sheet posted on the chambers' doors.

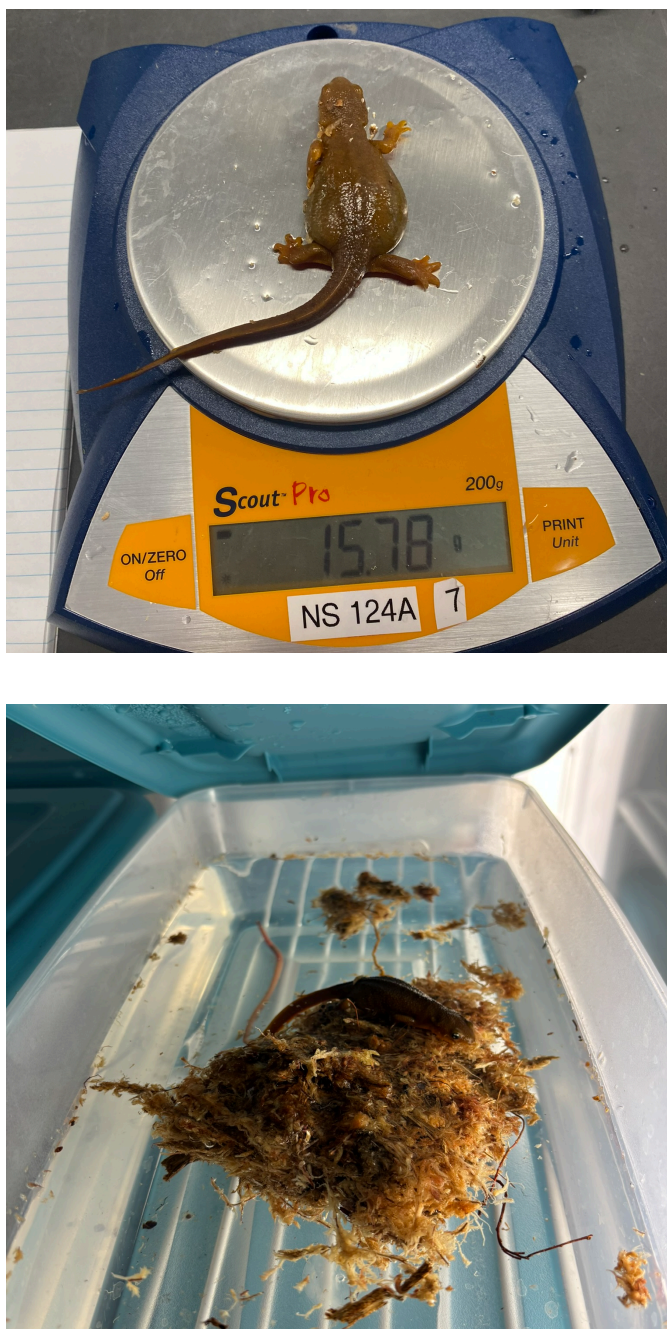


Fig. 7. Post-collection weighing (top) of female rough-skinned newts that were collected from Reynolds Creek/Placer Lake and Muddy Valley, as well as individual containers that all newts were placed in, marked with a unique identification number on the container to maintain data integrity (bottom).

Laboratory Methods - Experimental Tanks

Tank Set Up and Schedule

To observe possible preferential behavior towards varying salinity levels, multiple experimental tanks were created for a laboratory based experiment. Each tank offered a separate set of water treatments that newts were given the choice to interact with.

Experimental tanks consisted of four 20 gallon aquarium style tanks with a substrate of rehydrated sphagnum moss on top of rehydrated coconut shavings. The combined substrate was about three inches thick. Nestled into the substrate were two plastic containers. Each container measured 6" x 6" x 3" and was filled to the top (~1 litre). Water used in the tanks was pulled from the same de-chlorinated and de-ionized water that was used in the holding containers to reduce environmental change stress. Tanks were all placed on the same table with opaque dividers between shared glass walls to prevent newt-newt visual interactions and potential stress (Fig 8.).

The four tanks had four unique treatments set up with water of varying salinity based on average ranges found at Reynolds Creek and Placer Lake. The tanks consisted of (a) a tank with fresh water (0.00 ppt) and low salinity (0.50 ppt); (b) one tank with fresh water (0.00 ppt) and medium salinity (6.00 ppt); (c) a tank

with fresh water (0.00ppt) and high salinity (12.00 ppt); and (d) a tank with medium salinity (6.00 ppt) and high salinity (12.00 ppt). These tanks were arranged on a North-South orientation, with the lower salinity of the two available treatments in the given tank on the South-facing end, and the higher salinity placed on the North-facing side. This organization was presented as such to ensure that all hands removing newts and replacing materials were ensuring correct data for analysis later on.

Additionally, there is no peer-reviewed literature that points to a possible magnetic directionality when newts are navigating. Instead, there has been a vague determination that newts in the *Taricha* genus may use a combination of celestial cues, olfactory sense and kinesthetics to orient themselves and navigate terrain (Twitty 1966, Darrow 1967, Endler 1970). The animal holding facility is lit using direct overhead lights that do not change in direction or appearance during the day (except for on and off), so there are likely no additional influences from the laboratory lighting that would influence the directly that newts would travel within the tank.

Each tank was monitored using a Meidase S3 Pro Trail Camera on a tripod mount. These cameras were placed at one end of the tank and their directionality was marked in notes to ensure accurate data collection later on. Each camera was set to record for a total time of 24 hours, taking a burst of two photos every five minutes.

During the day, photos were taken in “true color” appearance, and black-and-white during the night period when the lights in the holding facility were off. Lights within the room mimicked the same schedule as the Percival Chambers to reduce stress from environmental change.

The 50 collected newts were randomly assigned to each treatment using a randomization algorithm. This was done to each individual population to ensure that an equal amount of newts from each population were assigned to each treatment ($n=6$ per population per treatment). Once selected, individual's containers were marked with their designated treatments. Each unique individual was placed in their assigned experimental tank for a period of 24 hours of recorded time. Newts were all placed in the middle of the tank on an area of substrate between the two water bodies and facing east for consistency throughout the project. During this time, they were not fed or handled to reduce possible interruption of baseline behavior. After their study period, newts were removed from the tanks and returned to their individual facilities. All water and substrate materials were changed between each 24 hour duration to prevent cross contamination and potential experiment disruption. All newts were only subjected to one treatment tank and were not reused to reduce exposure to stress or potential behavioral bias in the model.

After their original experimental treatments, newts were then randomly assigned to another set of treatments focusing on the physiological effects of long-term exposure to salinity. This experiment, which was not part of this thesis, was conducted over one month after their original treatment experiment to allow time for osmoregulation and stress levels to decrease. Prior to the beginning of this secondary test, newts were re-measured and weighed after a pat-dry to observe their weight before and after long-term exposure to salinity.



Fig 8. Experimental tank arrangement (a) and newt interaction(s) with the assigned variable treatment water (b, c). Each newt would spend 24 hours in one experimental tank and would not be assigned to another.

Data Analysis

Newt Morphometric Data Analysis

Morphometric data were separated into two categories, Snout-Vent-Length (SVL) and Total Length (TL). Data were tested for normality prior to analysis using a Shapiro-Wilk Test. Mean SVL and Total Length were compared between populations using independent two sample t-tests in R version 2025.9.2.418. SVL and TL were then represented on a ggerrorplot to visualize differences in the stated morphometric measurements.

Spatial Variation/Newt Size Distribution Mapping

Data for the spatial analysis of newt distribution in Reynolds Creek were collected from survey efforts. The data were formatted into a CSV file containing coordinates, salinity, depth, and temperature. Data were spatially analyzed using ArcGIS Pro and the collected data from the Reynolds Creek field survey(s). Data were then laid over a satellite image collected in 2022 as a part of the U.S. Geological Survey's National Agricultural Imagery Program (NAIP). Spatial data were then separated by tide using the Select Layer by Attribute tool. Each layer was attributed a single symbol, then assigned a unique color to distinguish the unique tidal groups.

Experimental Data Analysis

The photos collected from the laboratory experiment were recorded and analyzed to document time spent in water, time spent in either freshwater or saline water, time on land, and any additional unique behaviors that were exhibited. Photos were taken in a two set burst every five minutes, but were analyzed at 10 minute intervals due to the volume of photos. Photos were sorted into folders that matched each newt's unique ID and treatment. The photos from each newt were sorted based on the following criteria:

Time of the day

- a. Morning: 6:00 A.M. to 11:59 A.M
- b. Afternoon: 12:00 P.M. to 6:59 P.M.
- c. Night: 7:00 P.M. to 6:00 A.M.

In the water or on land

- d. Based on where the majority of their mass is situated (e.g. all body on land except for one limb categorized a “Land”)

Location in the tank

- e. Top or “North” side
- f. The middle
- g. The bottom or “South” side

Cardinal orientation based on head position

- h. North
- i. East

- j. South
- k. West

If the newt in the tank was not visually identifiable but the evaluator was confident that they were not in the water, they were marked as on land. If the newt was not visually identifiable and the evaluator was not confident that they were not in the water, the photo was “skipped”, and the observer would observe instead the photo at the next five minute interval. Each unique collection of photos had a number of 100 photos analyzed to ensure the same duration of observation was consistent for all newts in all treatments.

During the recording of these photos, water treatments were not labeled by their water characteristics, but rather by location in the tank based on cardinal directions. The photos were reviewed by a third party with no knowledge of the location of the controls and treatments within the tank. This allowed for a blind data collection process, reducing potential bias. The data from the third party recorder was then deblinded by the PI for data analysis.

Photo Data Analysis

Collected data from the experimental treatments were then organized and analyzed using R Studio (R Core Team (2025)). Prior to the statistical analysis, the data from each newt was compiled into a single CSV file. The file was then narrowed

down into a table containing variables ‘HALFOFTANK’, ‘TREATMENT’, ‘POPULATION’, ‘NEWT’, and ‘LOCATION’. Marginal totals were included in the table using ‘addmargins’. Instances with a zero-frequency or zero-observation were removed. The percentage of occupation on each side of the tank was calculated by dividing the value of the frequency of the newt on the side of the tank over the total frequency value. The data was then subset by treatment based on the value of the salinities (Fresh:Low, Fresh:Medium, Fresh:High, Medium:High).

After the data was subset by treatment type, it was then further subset into land occupation data points and water occupation data. Each subset of data (e.g., Fresh:Low; Land & Water) was analyzed as a separate entity to ensure accurate representations of time spent in land and water. A set of linear models, one for each treatment comparison, was then used to determine if there was a significant difference in percent of time spent in each half of the tank, using the explanatory variables HALFOFTANK and POPULATION and their interaction. Plots of the residuals were assessed to ensure that the data met the assumptions of the linear models.

Additionally, post hoc comparisons were completed with the emmeans package (Lenth R, Piaskowski J (2026)). Visualization of the data was created using visreg to depict models fits and the interactions of the variables. Using the psych

package, descriptive statistics were calculated. This process was also completed again by splitting the LOCATION variable into their respective outcomes, 'land' and 'water'. This process was then completed once more to analyze the difference in time spent on land and water for each salinity treatment variable.

Results

Spatial Salinity Variation

Large Scale Spatial Salinity Variation

At the Reynolds Creek water system, salinity varied greatly across the site of Reynolds' Creek and Placer Lake. Site lows were recorded at 0.06 ppt and highs of 25.28 ppt (Table 1). The site low was recorded over 0.25 kilometers from the source of freshwater located on the east side of the lake. Additionally, site high was located at the deepest part of Reynolds Creek, where it and Big Creek merge and flow out to the ocean. Despite the large volume of freshwater from Big Creek, the low depths of the delta read 25.28 ppt, where newts swam down to after disturbance from surveyors. The site saw a high frequency of a salinity value of 0.27 ppt, which is still classified as freshwater. This reading was seen across all four survey efforts within a 24 hour period.

Small Scale Salinity Variation

Salinity was found to vary within very small spatial scales. Placer Lake is fed by two small freshwater streams to the easternmost side of the body, each of which have a salinity of 0.07 ppt flowing into the lake. The lake however, has a salinity of 0.27 ppt. Less than four meters from the lake, a small, ephemeral puddle within ten meters from the two streams had a salinity of 8.20 ppt. Two recorded microhabitats within five meters from one another had a salinity ratio (ppt/meter) of 1.96 ppt/meter difference. Microhabitat A had a surface salinity of 0.30 ppt and a floor salinity of 3.00 ppt (1.5 meters deep). This gives Microhabitat A a ppt/m ratio of 2.70 ppt/m. Almost twice that, Microhabitat B had a salinity ratio of 4.66 ppt/m. Microhabitat B has a surface salinity of 0.27 ppt and a floor salinity of 4.00 ppt (1 meter deep) (Fig 9.).

Table 1. Summary statistics of survey points collected from the Reynolds Creek and Placer Lake water system, May 23rd and 24th, 2025. All included water characteristics were collected at sites of newt encounters. Each column represents one distinct tidal event during the 24 hour mixed semidiurnal tidal cycle.

	Overall	High Tide	Mi-High Tide	Mid-Low Tide	Low Tide
<i>Maximum (ppt)</i>	25.28	8.33	8.33	6.91	25.28
<i>Minimum (ppt)</i>	0.06	0.06	0.06	0.17	0.06
<i>Mean (ppt)</i>	1.62	2.37	0.58	0.92	1.73
<i>Mode (ppt)</i>	0.27	No Mode	No Mode	0.21	0.27



Fig 9. Two microhabitats within the Reynolds Creek water system, within five meters of one another. One location (A), had a salinity/meter ratio of 1.96 ppt/m where salinity increased with depth, whereas the other (B), despite being slightly shallower, had a salinity/meter ratio of 2.70ppt/m of salinity increasing with depth.

Temporal Salinity Variation

Small Scale Temporal Salinity Variation

Seen in the above table (Table 1), each tidal cycle experienced a different salinity maximum ranging from 6.91 to 25.28. Out of the four tidal events, three shared the same minimum of 0.06 ppt. Both the true high tide and the mid-high tide exhibited no mode in the documented salinities. The site maximum of salinity at newt encounters was 25.28 ppt, with a site average of 1.62 ppt and mode of 0.27 ppt. Within the same time frame during low tide, the water system experienced the widest range of salinity, from the highest recorded site salinity (25.28 ppt) to the freshest recorded site salinity (0.06 ppt), of 25.22 ppt. The mean recorded at each tidal event ranged from 0.58 ppt to 2.37 ppt.

Large Scale Temporal Salinity Variation

From the time of deployment to retrieval (Logger A May-September, Logger B June-September), both loggers experienced salinity change on a daily and monthly basis. Daily tidal fluctuations were accompanied by gradual monthly spikes in salinity.

These two loggers recorded different highs and lows at their deployment locations (measured in conductivity, mS/cm, but converted here to ppt for more consistent

interpretation). Seen in the following graphs and table, Logger A recorded higher salinity values than Logger B (Fig 10; Table 2). The westernmost logger, Logger A, had a difference of 17.48 ppt between the overall maximum and minimum values (22.33 ppt, 3.86 ppt). Logger B recorded a difference of 16.65 ppt between the overall maximum and minimum values (16.78 ppt, 0.09 ppt).

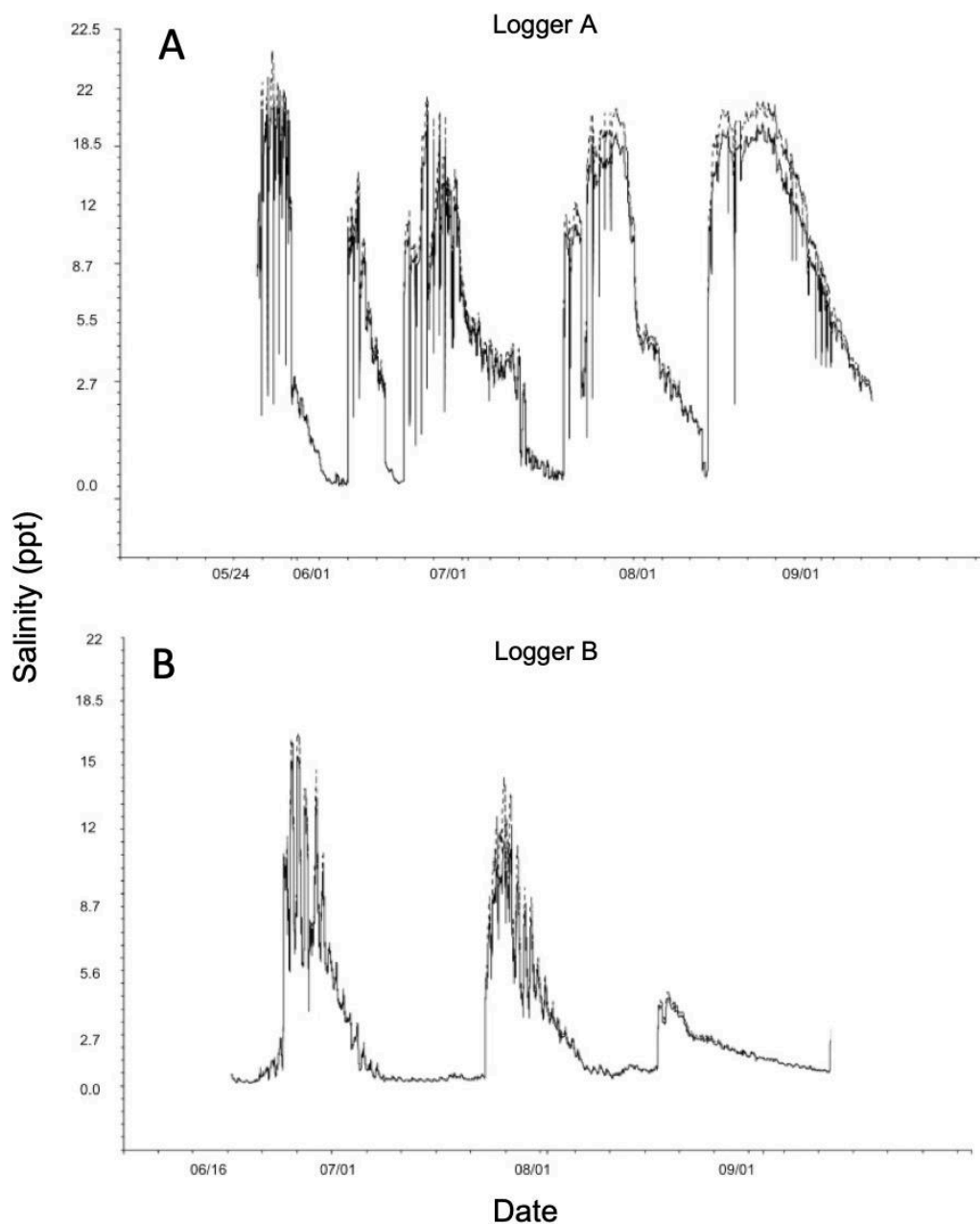


Fig 10. (a) The salinity range (ppt) from a) Logger A and b) Logger B. The solid line represents the High Range setting ($> \sim 72.2$ ppt) and the Low Range setting ($< \sim 5.6$ ppt) shown in the broken line, both of which were recorded simultaneously via HOBO Saltwater Conductivity Salinity Data Logger from the months of May to September 2025.

Table 2. Summary statistics of the High (>100 mS/cm) and Low (<10 mS/cm) Range setting's detection of conductivity (mS/cm) in Placer Lake (Logger A) and Reynolds Creek (Logger B) From the months of May (Logger A) and June (Logger B) to September of 2025.

	Range Setting	Maximum (ppt)	Minimum (ppt)	Mean (ppt)
Logger A	<i>High Range (>100 mS/cm)</i>	38.22	0.063	19.08
	<i>Low Range (<10 mS/cm)</i>	33.17	0.07	17.93
Logger B	<i>High Range (>100 mS/cm)</i>	30.05	0.28	6.80
	<i>Low Range (<10 mS/cm)</i>	25.74	0.28	5.83
	<i>Overall</i>	38.22	0.06	12.41

Spatial Distribution of Newts

Newts were observed at all of the previously mentioned salinity levels. Newts spanned salinities ranging from 0.06 ppt to 25.28 ppt. These two observations were documented in the same tidal cycle, during a “true” low tide event. Those in the 25.28 ppt area were originally seen in water reading 12.28 ppt, then swam to water of 25.28 ppt after disturbance, where they did not move for over five minutes.

Female rough-skinned newts were also observed ovipositing in varying salinities. While a visual majority of newts were located in the two freshwater streams feeding into Placer Lake, a few newts were ovipositing in the more saline parts of the water system. Newts were recorded ovipositing in a saline pond next to the streams (< 7 meters) in salinities of 8.20 ppt. The upper experimental salinity limit of an adult newt is 11 ppt, whereas an egg is only a fraction of that, at 4.80 ppt (Albecker et al 2026 unpublished data; determined based on laboratory experiments).

Newts were found to occupy a wide array of the Reynolds Creek site over the course of the 24 hour tidal cycle (Fig 11). Newts were closest to the ocean during the low tide and mid-high tide. Newts were observed as far west in the channel as the conjoining of Big Creek and as far east as the two freshwater streams feeding into Placer Lake. Aside from the densely populated eastern freshwater streams,

newts were seen the most in the channel of Reynolds Creek, between the joining of Big Creek and the head of Placer Lake.



Fig 11. Newt distribution throughout survey efforts in Reynolds Creek and Placer Lake (a) in May of 2025. Each dot color (b) represents a tidal cycle event, broken into true high tide (red), mid-high tide (orange), mid-low tide (green) and true low tide (blue). Dots may indicate more than one newt in each location as dots of multiple newts are overlapping.

Newt Morphology

Size and Sex Distribution

Of the observed male newts, the Snout-Vent-Lengths of the MVHR population were significantly larger than those in the Reynolds Creek population ($t=6.38$, $df=24.46$, $p<0.000001$). Observed MVHR males had a mean Snout-Vent-Length of 8.1 centimeters and a mean Total Length of 18.3 centimeters (Fig 13). Reynolds Creek males had a mean Snout-Vent-Length of 6.8 centimeters and a mean Total Length of 16.1 centimeters (Figs 13). This accounted for a 17.4% difference of Snout-Vent-Length means and a 12.8% difference of Total Length means between males of the two populations.

Much like the males, female newts' Snout-Vent-Lengths in the MVHR population were significantly larger than those of the Reynolds Creek population ($t=14.35$, $df=138.01$, $p<0.000001$). Of the observed newts, MVHR females had an average Snout-Vent-Length of 7.0 centimeters, and a mean Total Length of 15.8 centimeters (Fig 14). Reynolds Creek females had an average South-Venth-Length of 6.1 centimeters and a mean Total Length of 13.2 centimeters (Fig 14). There was a 13.7% difference of Snout-Vent-Length means, and an 18.7% difference of Total Length means.

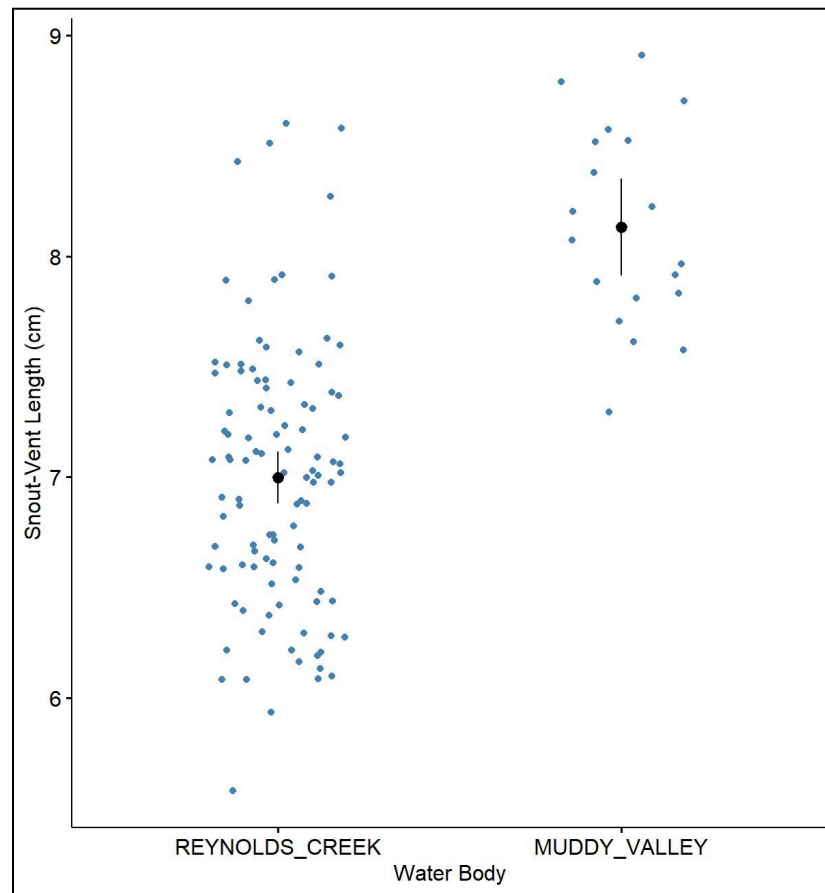


Fig 13. Differences between the Snout-Vent Length (SVL) in male Rough-skinned newts from Muddy Valley ($n= 19$) and Reynolds Creek ($n= 104$), surveyed and collected in May 2025. Black dots represent the mean SVL of each population, with a 95% confidence interval represented as a black line. Each blue dot represents one point of data from an individual newt.

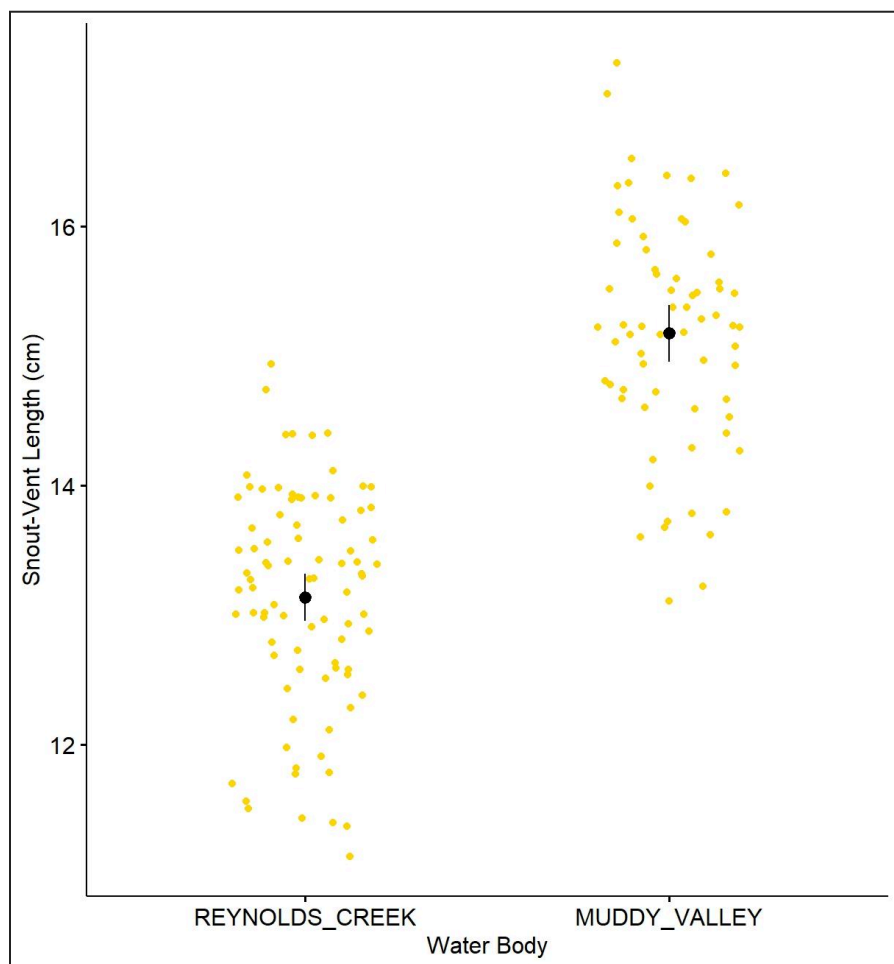


Fig 14. Differences between the Snout-Vent Length (SVL) of female Rough-skinned newts from Muddy Valley ($n= 67$) and Reynolds Creek ($n= 86$), surveyed and collected in May 2025. Black dots represent the mean SVL of each population, with a 95% confidence interval represented as a black line. Each yellow dot represents one point of data from an individual newt.

Laboratory Results

Time spent in each side of the tank

Fresh vs Low

In the Fresh:Low (0.0 ppt, 0.5 ppt) treatment tank, newts from both populations favored the low salinity side, spending more of their time in that area of the tank than any other area ($F=6.382$, $df=2, 27$, $p=0.005$). There was, however, no difference in the time spent in the fresh, middle, or low salinity areas averaged across both populations ($F=3.057$, $df=1,27$, $p=0.542$), and there was not a significant difference in tank usage averaged across populations ($F=0.457$, $df=2, 27$, $p=0.637$) (Table 3).

Muddy Valley newts favored the low salinity side over the fresh and middle areas, spending 55 percent (± 12.3) of their time in the low salinity area (Fig 15). This was significantly larger than the percent of time spent in the middle area of the tank ($t= -2.606$, $df=27$, $p=0.038$). There was, however, no significant difference in the time spent between the fresh and low salinity sides of the tank ($t= -1.13$, $df=27$, $p=0.504$) or in the time spent between the fresh water and middle areas of the tank ($t= 1.475$, $df= 27$, $p= 0.318$). Reynolds Creek spent an almost even percent of time in the fresh water area (49.40 ± 13.5) and the low salinity area (46.00 ± 13.5) of the tank, with a non-significant difference between the percent of time spent in either area ($t= 0.178$, $df=27$, $p= 0.982$) (Fig 15). There was an approaching statistical

significance between the percent of time spent in the fresh water area over the middle area ($t = 2.351$, $df = 27$, $p = 0.066$), but there was no significant difference between time spent in the low salinity and middle areas of the tank ($t = -2.172$, $df = 27$, $p = 0.094$).

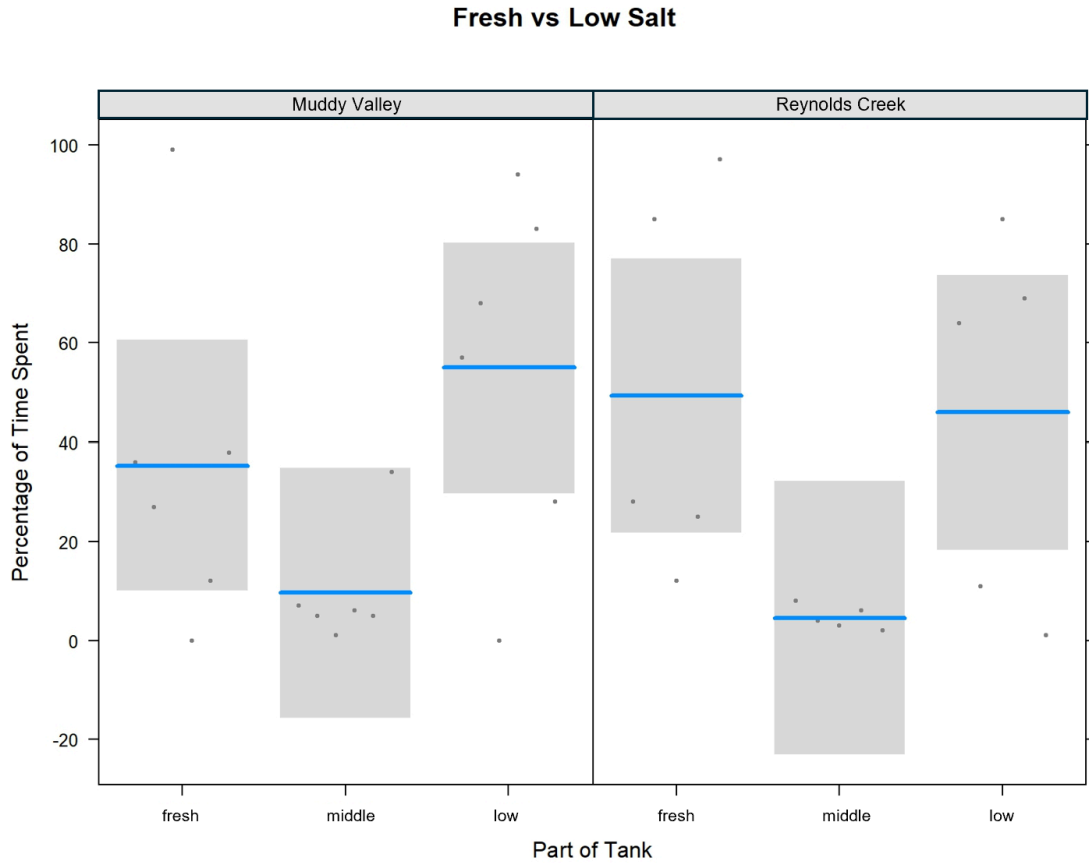


Fig 15. Time spent in each treatment area of the Fresh vs Low Salt treatment tank represented in percentage of occupation, separated by population. Percentage of time is represented on the Y axis, with treatment area (fresh, salt, middle) shown along the X axis. Blue lines represent the mean time spent in each area, with each grey dot depicting a percentage of time from an individual newt. Grey boxes represent the 95% confidence intervals.

Fresh vs Medium

In the Fresh:Medium (0.0 ppt, 6.0 ppt) treatment tank, newts from both populations favored the fresh water side of the tank over other areas, though not significantly ($F= 2.736$, $df= 2,30$, $p= 0.081$). There was an approaching, non-significant difference between time spent in the differing areas between the populations, with Muddy Valley newts spending $52.5 (\pm 8.73)$ percent of their time in the freshwater area, and Reynolds Creek newts spending only $35.5 (\pm 8.73)$ percent of their time in the freshwater area ($F= 2.736$, $df= 2,30$, $p= 0.081$). There was no overall difference between the time spent in either area of the tank, averaged across populations ($F= 1.523$, $df= 2, 30$, $p= 0.234$) (Table 3)

Muddy Valley newts spent over half of their time in the fresh water side of the Fresh:Medium tank (52.5 ± 8.73), a significantly larger portion of time than what was spent in the middle area of the tank ($t= 2.820$, $df= 30$, $p= 0.022$) (Fig 16). There was, however, no significant difference in time spent between the freshwater and medium salinity sides of the tank ($t=1.846$, $df=30$, $p=0.172$), nor was there a significant difference between the middle and medium salinity areas ($t= -0.974$, $df= 30$, $p= 0.599$). Unlike Muddy Valley newts, the Reynolds Creek population only spent a very slight majority of the time in the medium salinity area of the tank (35.4 ± 8.73) over the middle (29.3 ± 8.736) and the freshwater area (35.3 ± 8.73) of the tank (Fig 16). With this somewhat even split in percent of time split, there was no significant difference in percent of time between the freshwater and middle

areas ($t = 0.481$, $df = 30$, $p = 0.881$). Similarly, there was no statistical difference between the percent of usage of the freshwater and medium salinity sides ($t = -0.008$, $df = 30$, $p = 1.0$), nor between the middle and medium salinity areas of the tank ($t = -0.974$, $df = 30$, $p = 0.877$).

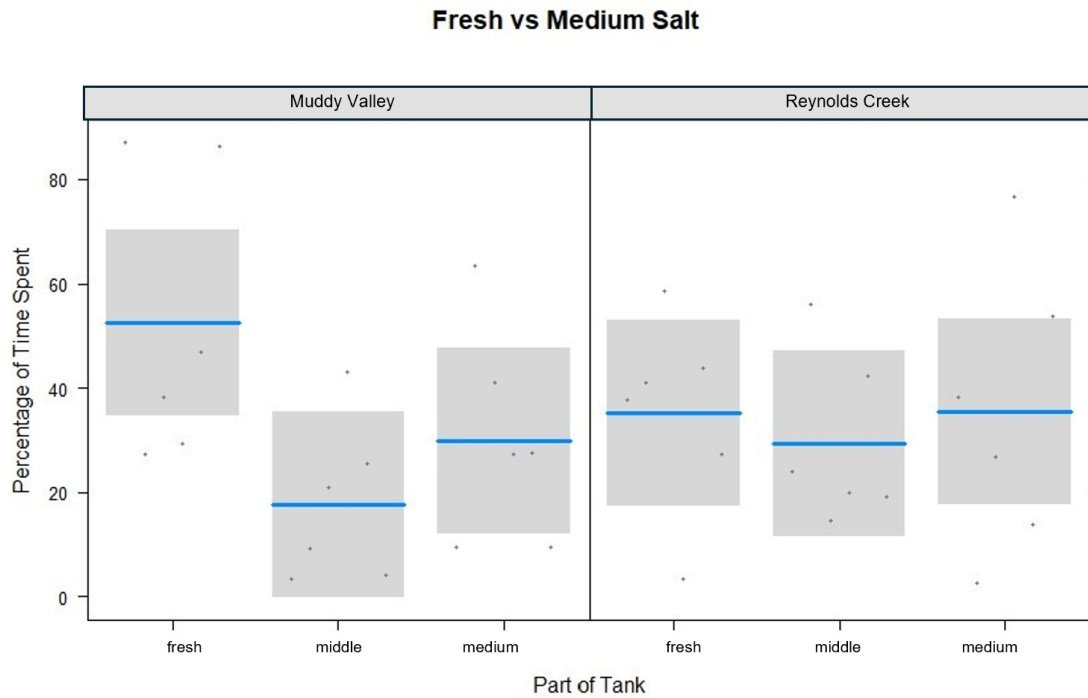


Fig 16. Time spent in each treatment area of the Fresh vs Medium Salt treatment tank represented in percentage of occupation. Percentage of time is represented on the Y axis, with treatment area (fresh, salt, middle) shown along the X axis. Blue lines represent the mean time spent in each area, with each grey dot depicting a percentage of time from an individual newt. Grey boxes represent the 95% confidence intervals.

Fresh vs High

In the Fresh:High (0.0 ppt, 12.0 ppt) treatment tank no significant difference in time was spent in any of the tank areas between either population ($F = 0.309$, $df = 2, 27$, $p = 0.737$). Additionally, no significant difference was found between the areas themselves ($F = 0$, $df = 1, 27$, $p = 1$). There was also no significant difference in the percent of usage, averaged between the two populations ($F = 0.989$, $df = 2, 27$, $p = 0.349$) (Table 3).

Much like their Fresh:Medium counterparts, Muddy Valley newts in the Fresh:High treatment tank spent the majority of their time in the freshwater area of the tank (50.1 ± 13.3) (Fig 18). This was not significantly different from the time spent in the high salinity water ($t = 1.174$, $df = 27$, $p = 0.4784$), or between the freshwater and middle areas ($t = 1.493$, $df = 27$, $p = 0.310$). Additionally, the time spent between the middle and high salinity areas of the tank was also not significantly different ($t = -0.318$, $df = 27$, $p = 0.946$). In the Reynolds Creek newts, time spent in the high salinity area of the tank (31.9 ± 10.1) was barely higher than the time spent in the freshwater area of the tank (31.8 ± 10.1), but both found themselves less occupied than the middle area of the tank (36.2 ± 10.1) (Fig 17). With such even area usage, the percent of time in the freshwater area did not differ significantly from the middle area of the tank ($t = -0.309$, $df = 27$, $p = 0.949$), nor did it differ significantly from the time spent in the high salinity water area ($t = -0.004$, $df = 27$, $p = 1$). Additionally, the percent of time spent between the high

salinity area and the middle area did not differ significantly ($t= 0.305$, $df= 27$, $p= 0.905$).

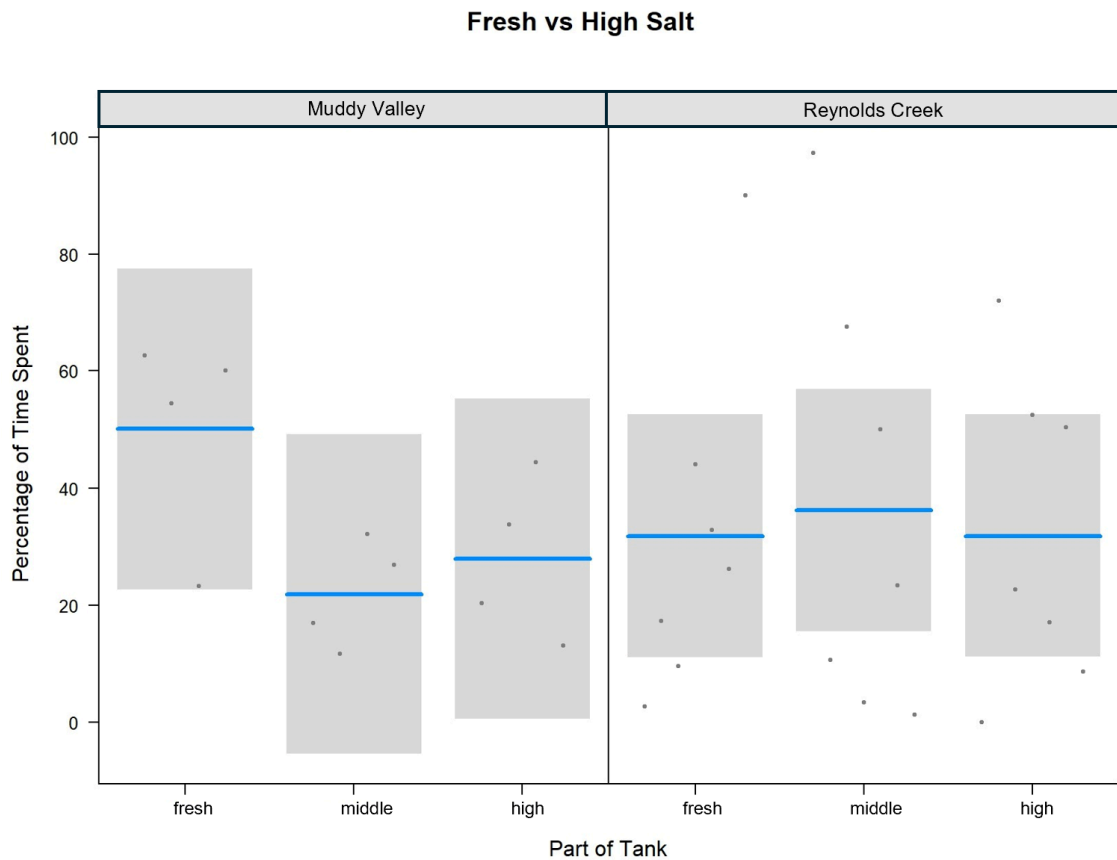


Fig 17. Time spent in each treatment area of the Fresh vs High Salt treatment tank represented in percentage of occupation. Percentage of time is represented on the Y axis, with treatment area (fresh, salt, middle) shown along the X axis. Blue lines represent the mean time spent in each area, with each grey dot depicting a percentage of time from an individual newt. Grey boxes represent the 95% confidence intervals.

Medium vs High

In the Medium:High (6.0 ppt, 12.0 ppt) treatment tank, both populations favored medium salinity areas, spending a significantly larger portion of their time in that area ($F = 6.84$, $df = 2, 27$, $p = 0.005$). There was no significant difference in percent of usage between populations, with both spending nearly half of their time in the medium salinity side ($F = 0$, $df = 1, 27$, $p = 1$). Similarly, there was not a significant difference in usage of the tank, averaged across populations ($F = 0.313$, $df = 2, 27$, $p = 0.735$).

Muddy Valley newts slightly favored the medium salinity area of the tank, spending a small majority of their time in that area of the tank (48.09 ± 13.3) (Fig 18). This area was significantly larger than the time spent in the middle area ($t = 2.144$, $df = 27$, $p = 0.099$), as well as the high salinity area of the tank ($t = 0.201$, $df = 27$, $p = 0.978$). In a similar comparison, the Muddy Valley newts did not spend a significant different percent of time between the high salinity and middle areas of the tank ($t = -1.943$, $df = 27$, $p = 0.146$). Reynolds Creek newts also favored the medium salinity water over the other available tank areas (48.95 ± 14.6) (Fig 18). This was not significantly higher or lower than the percent of time spent within the middle area of the tank ($t = 1.4456$, $df = 27$, $p = 0.333$) or the high salinity side of the tank ($t = 0.820$, $df = 27$, $p = 0.694$). The time spent in the high salinity side of the tank, while higher than the time spent in the middle area (31.98 ± 14.6 ,

19.07±14.6), they were not significantly different from each other ($t = -0.624$, $df = 27$, $p = 0.808$).

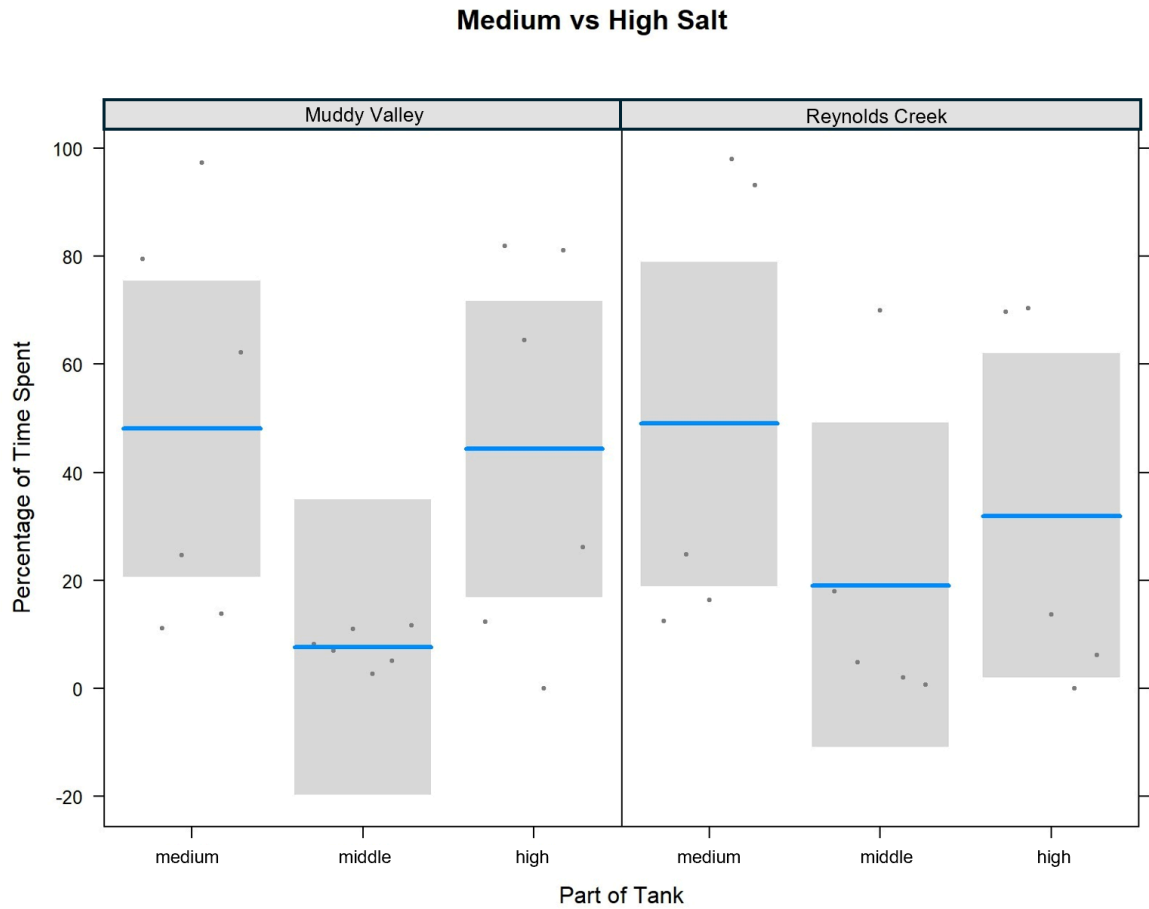


Fig 18. Time spent in each tank area of the Medium vs High Salt treatment tank represented in percentage of occupation. Percentage of time is represented on the Y axis, with treatment area (fresh, salt, middle) shown along the X axis. Blue lines represent the mean time spent in each area, with each grey dot depicting a percentage of time from an individual newt. Grey boxes represent the 95% confidence intervals.

Table 3. Results from linear models examining the effects of tank area, newt population, and their interaction on percentage of time spent over 24 hours in a particular area of the tank, combining water and land. Each treatment comparison is a separate model. Muddy Valley newts n=22, Reynolds Creek newts n=23.

Treatment Comparison	Main Effect	F (df)	P				
Fresh – Low	Tank Area	3.057 (2,27)	0.542		Mean±SE % Time in Fresh	Mean±SE % Time in Middle	Mean±SE % Time in Salt
	Population	6.38 (2, 27)	0.005	Muddy Valley	35.33±12.3	9.67±12.3	55.00±12.3
	Tank Area x Population	0.457 (2, 27)	0.637	Reynolds Creek	49.40±13.5	4.60±13.5	46.00±13.5
Fresh – Medium	Tank Area	0 (1,30)	1		Mean±SE % Time in Fresh	Mean±SE % Time in Middle	Mean±SE % Time in Salt
	Population	2.736 (2,30)	0.081	Muddy Valley	52.5±8.73	17.7±8.73	29.7±8.73
	Tank Area x Population	1.523 (2,30)	0.234	Reynolds Creek	35.3±8.73	29.3±8.73	35.4±8.73
Fresh – High	Tank Area	0 (1,27)	1		Mean±SE % Time in Fresh	Mean±SE % Time in Middle	Mean±SE % Time in Salt
	Population	0.309 (2,27)	0.737	Muddy Valley	50.1±13.3	22.0±13.3	28.0±13.3
	Tank Area x Population	0.989 (2,27)	0.349	Reynolds Creek	31.8±10.1	36.2±10.1	31.9±10.1
Medium – High	Tank Area	6.84 (2,21)	0.005		Mean±SE % Time in Fresh	Mean±SE % Time in Middle	Mean±SE % Time in Salt
	Population	0 (1,27)	1	Muddy Valley	48.09±13.3	7.62±13.3	44.29±13.3
	Tank Area x Population	0.313 (2,27)	0.735	Reynolds Creek	48.95±14.6	19.07±14.6	31.98±14.6

Time Spent in Each Water Body

Fresh vs Low

In the Fresh:Low treatment tank (0.0 ppt, 0.5 ppt), there was not a water treatment that was occupied more significantly than the other ($F = 0.741$, $df = 1, 16$, $p = 0.402$). There was also not a significant difference in the percent of usage from either populations ($F = 0$, $df = 1, 16$, $p = 1$), nor was the usage of either water treatment different, averaged across populations ($F = 0.016$, $df = 1, 16$, $p = 0.899$) (Table 4).

Muddy Valley newts spent the majority of their time in the low salinity water (57.1 ± 17.1) and less time in the freshwater (42.9 ± 17.1). These percent differences, however, were not statistically significantly different from one another ($t = -0.586$, $df = 16$, $p = 0.566$) (Fig 19). This pattern was closely reflected in the Reynolds Creek population where, despite the majority of the time being spent in the low salinity water (59.5 ± 20.9) over the freshwater (40.5 ± 20.9), there was not a significant difference between the two usage percents ($t = -0.643$, $df = 16$, $p = 0.529$) (Fig 19).

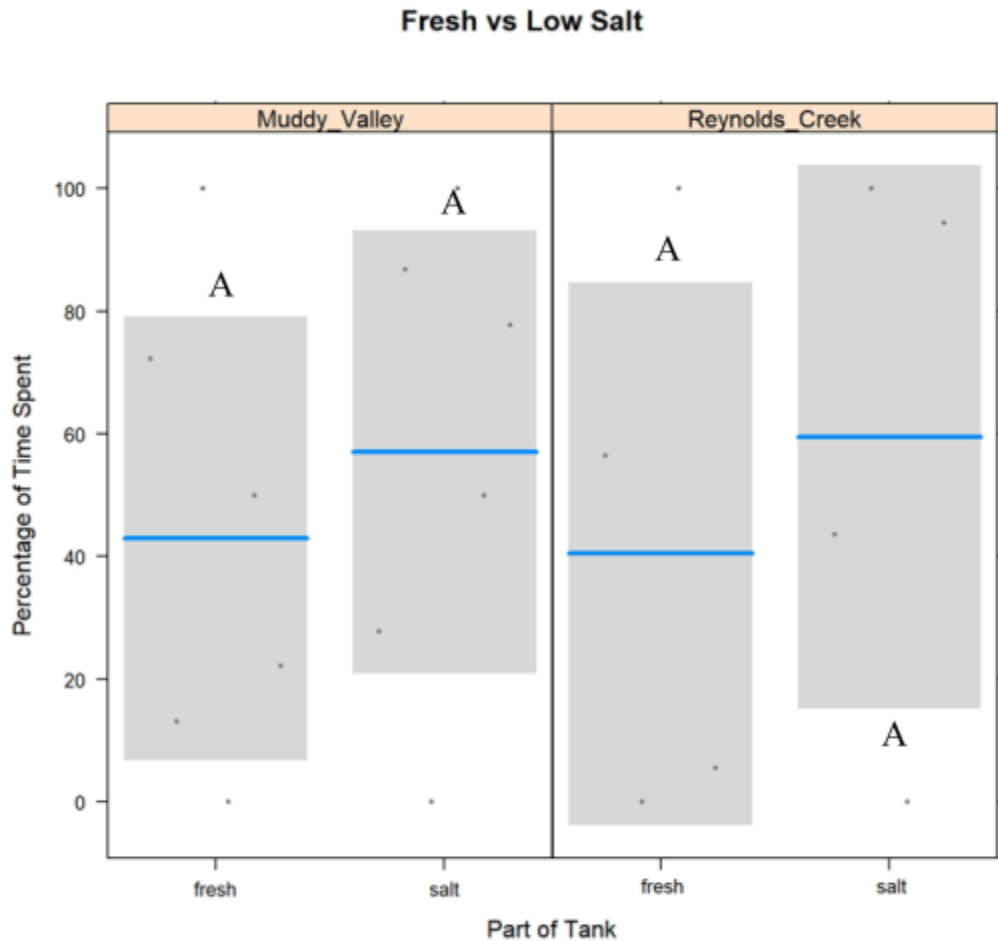


Fig 19. Percent of time spent in each area of the Fresh vs Low Salt treatment tank over 24 hours, by female newts from Muddy Valley (n=6; left panel) and Reynolds Creek (n=4; right panel). Blue lines represent the mean time spent in each area, with each grey dot depicting a raw data point from one newt. Grey boxes represent the model-predicted 95% confidence interval (CI). Different letters above means indicate Tukey-adjusted significant differences.

Fresh vs Medium

In the Fresh:Medium treatment tank (0.0 ppt, 6.0 ppt), there was an approaching, non-significant difference in time spent in either water treatment ($F= 3.760$, $df= 1$, 18 , $p= 0.068$). Additionally, there was no significant difference in the population's use of the water treatments ($F= 0$, $df= 1$, 18 , $p= 1$), as well as usage percentages of each water treatment, averaged across populations, though there was it was nearing statistical significance ($F= 4.066$, $df= 1$, 18 , $p= 0.0590$) (Table 4).

Muddy Valley newts in the Fresh:Medium treatment tank spent the majority of their time in the freshwater treatment (77.2 ± 13.8), more than double the time spent in the medium salinity water (22.7 ± 13.8). The amount of time spent in the freshwater was significantly larger than the time spent in the medium salinity water ($t= 2.792$, $df= 18$, $p= 0.012$) (Fig 20). In Reynolds Creek newts, there was not a large difference in time spent between the freshwater (48.1 ± 15.1) and the medium salinity water (51.9 ± 15.1). Unlike the Muddy Valley population, the difference between water treatment usage here was not statistically significant ($t= -0.182$, $df= 18$, $p= 0.858$) (Fig 20).

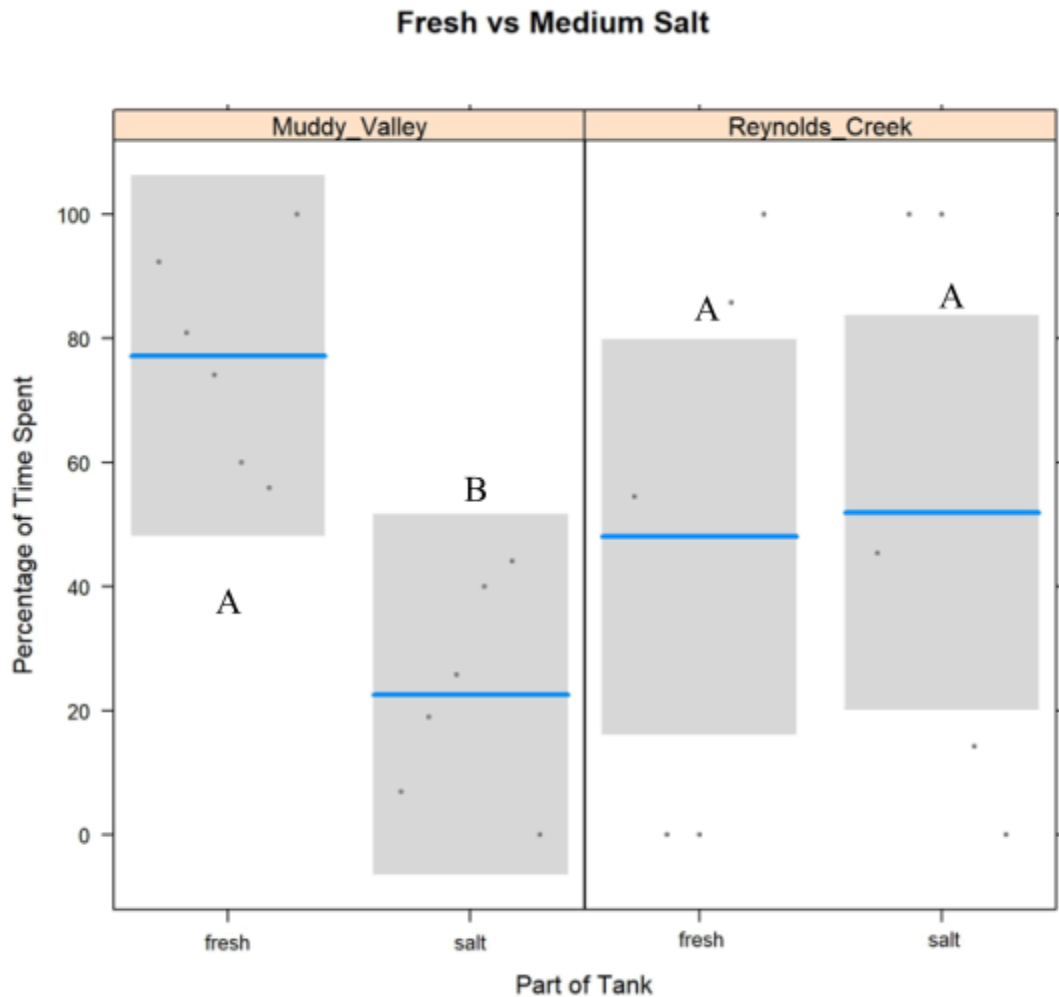


Fig 20. Percent of time spent in each area of the Fresh vs Medium Salt treatment tank over 24 hours, by female newts from Muddy Valley (n=6; left panel) and Reynolds Creek (n=5; right panel). Blue lines represent the mean time spent in each area, with each grey dot depicting a raw data point from one newt. Grey boxes represent the model-predicted 95% confidence interval (CI). Different letters above means indicate Tukey-adjusted significant differences.

Fresh vs High

In the Fresh:High treatment tank (0.0 ppt, 12.0 ppt), there was a significant difference in the percent of time spent in either available water treatment, with both populations spending the majority of their time in the fresh water of the tank ($F= 6.033$, $df= 1, 18$, $p= 0.024$). There was not a significant difference in the percent of occupation from population to population ($F= 0.213$, $df= 1, 18$, $p= 0.118$), nor was the usage of the two water treatments significantly different from one another, averaged across the two populations ($F= 2.701$, $df= 1, 18$, $p= 0.118$) (Table 4).

Muddy Valley newts spent nearly three-quarter of their time in the fresh water in the tank (74.0 ± 15.6). This was significantly more time than what was spent in the high salinity water in the experimental tank ($t= 2.792$, $df=18$, $p= 0.012$) (Fig 21). Similarly, Reynolds Creek newts spent over half of their time in the Fresh:High tank in the available freshwater (57.7 ± 11.8). Unlike their counterparts, however, this was not a significantly different percent of time than what was spent in the high salinity water in the tank (41.6 ± 11.8) ($t= 0.968$, $df= 18$, $p= 0.354$) (Fig 21).

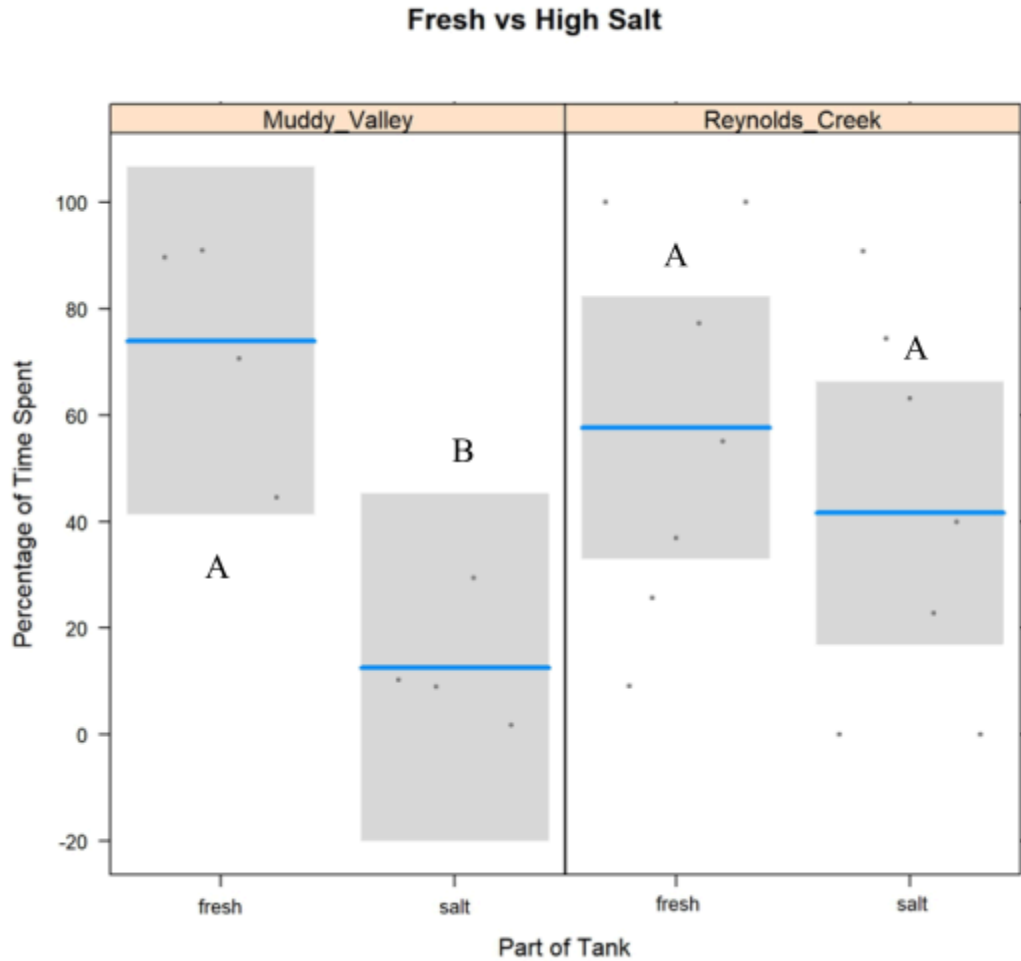


Fig 21. Percent of time spent in each area of the Fresh vs High Salt treatment tank over 24 hours. by female newts from Muddy Valley (n=4; left panel) and Reynolds Creek (n=7; right panel). Blue lines represent the mean time spent in each area, with each grey dot depicting a raw data point from one newt. Grey boxes represent the model-predicted 95% confidence interval (CI). Different letters above means indicate Tukey-adjusted significant differences.

Medium vs High

In the Medium:High treatment tank (6.0 ppt, 12.0 ppt), there was not a significant difference between the time spent in the high salinity and medium salinity water within the tank, with both populations splitting their usage nearly evenly ($F=0.084$, $df=1, 14$, $p=0.777$). Similarly, there was not a significant difference in the usage patterns within each population ($F=0$, $df=1, 14$, $p=1$), nor was there when usage was averaged across populations ($F=0.419$, $df=1, 14$, $p=0.529$) (Table 4).

Muddy Valley newts, despite prior treatment tank trends, spent the majority of their water usage in the high salinity water (52.8 ± 18.2), though it was not significantly more than the time spent in the medium salinity water that was available in the tank (47.2 ± 18.2) ($t=-0.215$, $df=14$, $p=0.833$) (Fig 22). Reynolds Creek newts had a similar even split of time between the medium and high salinity water, but instead slightly favored the medium salinity (59.7 ± 20.4) over the high salinity water (40.3 ± 20.4). Much like Muddy Valley newts, this difference in percent of usage of either water treatment was not significantly larger than each other ($t=0.674$, $df=14$, $p=0.511$) (Fig 22).

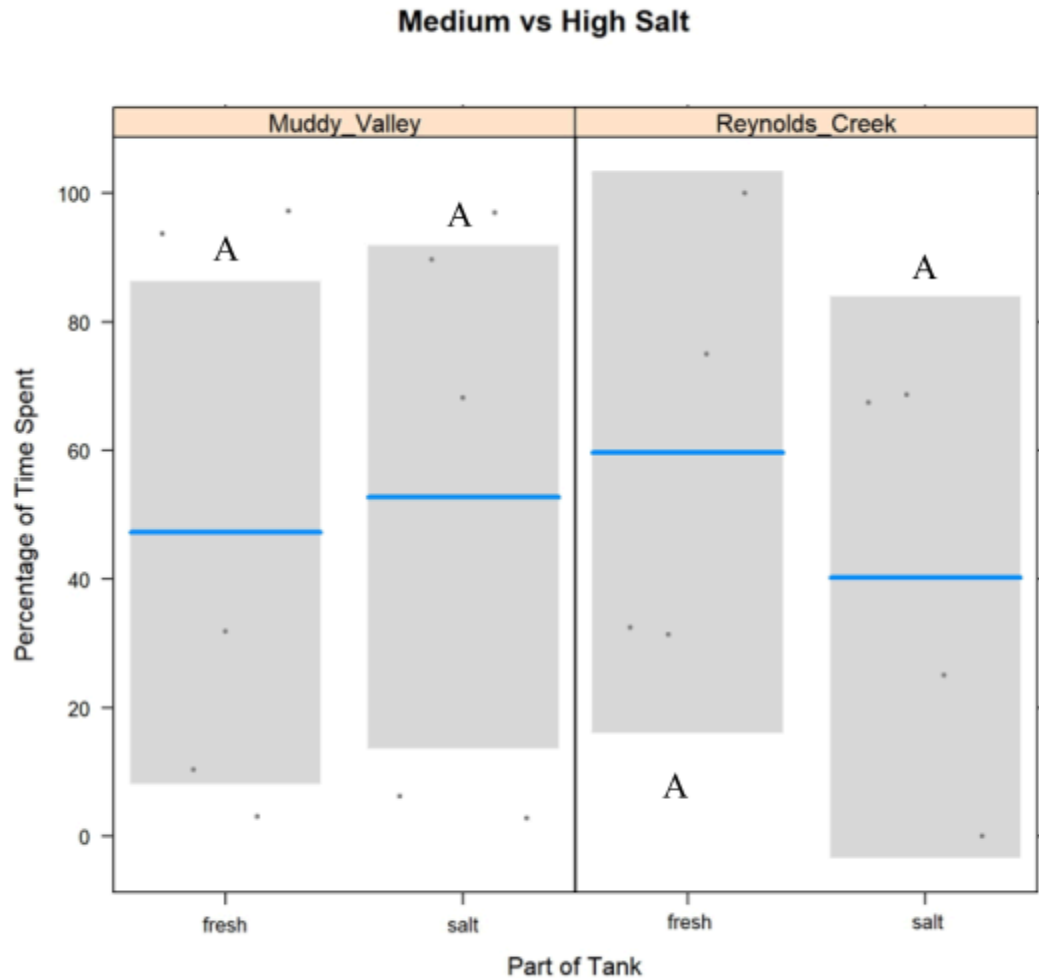


Fig 22. Percent of time spent in each area of the Medium vs High Salt treatment tank over 24 hours. by female newts from Muddy Valley (n=5; left panel) and Reynolds Creek (n=4; right panel). Blue lines represent the mean time spent in each area, with each grey dot depicting a raw data point from one newt. Grey boxes represent the model-predicted 95% confidence interval (CI). Different letters above means indicate Tukey-adjusted significant differences.

Table 4. Results from linear models examining the effects of tank area, newt population, and their interaction on percentage of time spent over 24 hours in a particular area of the tank, solely in the provided water treatment(s). Each treatment comparison is a separate model. Muddy Valley newts n=22, Reynolds Creek newts n=23.

Treatment Comparison	Main Effect	F (df)	P			
Fresh – Low	Tank Area	0.741 (1,16)	0.402		Mean±SE % Time in Fresh	Mean±SE % Time in Salt
	Population	0 (1,16)	1	Muddy Valley	42.9±17.1	57.1±17.1
	Tank Area x Population	0.016 (1,16)	0.899	Reynolds Creek	40.5±20.9	59.5±20.9
Fresh – Medium	Tank Area	3.760 (1,18)	0.068		Mean±SE % Time in Fresh	Mean±SE % Time in Salt
	Population	0 (1,18)	1	Muddy Valley	77.2±13.8	22.7±13.8
	Tank Area x Population	4.066 (1,18)	0.059	Reynolds Creek	48.1±15.1	51.9±15.1
Fresh – High	Tank Area	6.033 (1,18)	0.024		Mean±SE % Time in Fresh	Mean±SE % Time in Salt
	Population	0.212 (1,18)	0.650	Muddy Valley	74.0±15.6	12.6±15.6
	Tank Area x Population	2.701 (1,18)	0.118	Reynolds Creek	57.7±11.8	41.6±11.8
Medium – High	Tank Area	0.084 (1,14)	0.777		Mean±SE % Time in Medium	Mean±SE % Time in High
	Population	0 (1,14)	1	Muddy Valley	47.2±18.2	52.8±18.2
	Tank Area x Population	0.417 (1,14)	0.529	Reynolds Creek	59.7±20.4	40.3±20.4

Discussion

Salinity Variation

When characterizing salinity in a habitat, knowing the salinity of a source water body and the body it is encroaching on is not enough data to precisely and accurately predict the way that it will be displayed across the original water system. Salinity within ecosystems varies on a scale far more complex than previously detailed. Even within a small habitat like Reynolds Creek, salinity varies greatly across the site, as well as within just a meter between measurements. Complex structures and dynamic water beds can create unique pockets of stagnation in a system. This can also be caused by large rocks, pits, downed trees, and animal usage. Rivets and channels of varying size can have an opposite effect. With areas of high flow or open water pathways, high mixing can occur, creating portions of even salinity. The shape and structure of an ecosystem can also affect the path and quantity of salinity in a given area (Banmari et al. 2021).

As seen with the long-term monitoring of salinity at Reynolds Creek, two loggers within the same water body can experience different salinity values, as well as different timing of salinity changes. The logger closest to the ocean documented more frequent and more drastic changes in salinity, likely due to its close proximity to the ocean and incoming water. The logger further inland, at the

easternmost side of the lake, experienced slightly lower salinity overall, and had less extreme spikes in salinity, with roughly one large spike occurring every month. This is likely due to geographic variation, as well as the monthly moon cycle, which brings in higher tides and a stronger influx of salinity (Garret & Maas, 1993). Indeed, the largest salinity values corresponded to high tide on dates with full moons (Fig 10). Additionally, knowing the salinity and its variance in one habitat does not dictate the behavior of salinity in surrounding habitats and similar ecosystems. This behavior, while likely to occur at other locations, will not necessarily create the same values as seen at this study site. Each location has its own geographic characteristics, environmental factors, and biodiversity that influence the salinity of that site.

All of these seemingly inconsequential factors all compound on one another to create an incredibly complex environment for amphibians in the area to navigate. Rough-skinned newts in the Reynolds Creek water system can experience water anywhere from 0.06 ppt to 25.28 ppt, if not higher. These values don't just infiltrate in a predictable, uniform manner. They sneak into ruts and pockets of the ecosystem, creating seemingly random high stress environments. Newts and other amphibians in the area must skillfully navigate this habitat to avoid long term exposure to potentially disadvantageous environments. That said, the likelihood that the local newt population can withstand, at least temporarily, higher salinities

than previously recorded is a strong possibility, with newts being observed in salinities over 25 ppt.

Saline Environment Classification

With the above in mind, it is apparent that the organization and classification of salinity in habitats needs to be reexamined and reclassified. There are a handful of key factors to consider when observing a location and the behavior of the water and salinity in that given location.

Measuring the frequency of different salinities in an environment is crucial to understanding the level of salinity that the habitat experiences in a given time frame. This salinity may come from many different sources, such as anthropogenic, tidal/ocean, substrate, and vegetation (Neitsch et al., 2011). If the environment is experiencing frequent salinity influxes, there may be a higher likelihood that the habitat will have more prolonged areas of salinity within the system. Taking Reynolds Creek as an example, experiencing tidal influxes twice in a 24 hour time frame indicates that there are at least two periods where salinity will rise in the system. This is one way a habitat can experience high salinity. Seasonal droughts or intermittent weather patterns can also change the frequency in which a system experiences salinity.

Another factor to examine when characterizing a site is the timing of the salinity intrusion. The time of salinity influxes may influence the activity of the organisms in the environment. Tidal influxes during the day may affect diurnal species' movements, whereas nightly salinity fluctuations may affect those who are nocturnal. The long-term timing of salinity may also impact the distribution and physiology of organisms within the habitat (Rubega & Robinson, 1997). Salinity increases may not pose an osmotic risk to those who are not in the area at the time (i.e. an increase in salinity in the winter would not pose a risk to organisms out of the water and/or area during the winter months). This seasonal change may also affect the overall characteristics of the water body and the way that it is classified on a year-round basis.

Just as important as timing and frequency of salinity influxes, is the predictability of these influxes. Predictability allows for organisms to potentially adapt to the environment based on regularity of the changes in salinity. Many coastally located areas experience salinity in a fairly predictable way. Tides are regular and the severity is well known, so the level of saline water that will encroach is measurable. Organisms in the ecosystem can either withstand the changes in salinity, or remove themselves from the stress habitat (or they will perish). Other habitats have constant and predictable salinity, such as constantly saline lakes. These habitats demand osmotic tolerance from their permanent residents. There are, however, a number of habitats and ecosystems that exist between these two

examples. Salinity may change in these environments for a number of reasons, whether it be tidal, storm surge, anthropogenic, or other (Chaieb et al., 2019). Disturbances such as these may be frequent or infrequent, but their unpredictability creates an environment that is difficult to monitor and organisms to adapt.

The reality is that many organisms are living in habitats of unpredictable salinity changes on a scale ranging from frequent to infrequent exposure. That said, relevant research is rarely conducted in these habitats, therefore, they are being ascribed to the textbook classifications of freshwater, saltwater, and brackish water, as seen in European Nature Information System, EUNIS, which uses strictly the labels above (Davies, 2000) . This method is well known, but outdated when trying to understand the behavioral dynamics of sensitive species, like amphibians. To properly observe, characterize, and analyze complex amphibian behaviors, more intention must first be devoted to understanding their habitats. Documenting the frequency, timing, and predictability of a habitat creates more observational depth, allowing for a more comprehensive and complete understanding of the environment that species are facing in day-to-day and season-to-season scenarios (Li et al., 2013).

Newt Morphometrics

Despite being the same species, newts between the inland MVHR and the coastal Reynolds Creek populations are significantly different in morphology, with Reynolds Creek newts much smaller than those of their inland counterparts. While underlying population genetics may play a role in this size difference, there is also evidence that points to an environmental factor, like salinity, having an impact of final size at maturation. The hypothesis that exposure to salinity during the life cycle of an amphibian may result in a smaller end size could be prevalent here (Tornabene et al., 2019). This may be the result of an energy cost trade-off that occurs when these species are in osmotically stressful environments for longer than ideal times (Lorrain-Soligon et al., 2023). The energetic cost of maintaining osmoregulatory balance may require more energy. If the energy cannot be maintained through external consumption or other means, the newt may pull on energy stored for body growth and instead utilize it to keep themselves alive. Faster maturation and smaller end size may also be a means to gain mobility and remove themselves from this disadvantageous environment (Lorrain-Soligon et al., 2023).

A similar pattern has been observed on the coast of France, where Spadefoot toads (*Pelobates cultripes*) who lived on the coast and were exposed to salinity had a smaller overall size in both mature females and males, compared to their relatives

living in constantly freshwater ponds. This observation has also been hypothesized to be a result of the osmotic stress that occurs in the coastally located ponds (Lorrain-Soligon et al., 2022). This significant difference is mirrored in the MVHR and Reynolds Creek populations, who also display strong morphometric differences between the two populations.

Due to the scope of the project, vegetation and prey availability, as well as local climate, were not measured as direct factors in this observation. Nutrient availability, genetic diversity, predator status, and prey availability may also play a role in the success and size of the newts within the Reynolds Creek water system. The unpredictability of salinity, coupled with sparse vegetation and invertebrate populations may negatively impact newts' feeding, breeding, and maturation (Lorrain-Soligon et al., 2024). Certain environmental factors may also unevenly affect amphibians and their morphology based on the nature of the stressor (Lewis et al., 2021). These all remain important future areas of study to investigate.

Experimental Laboratory Behavior

In the Fresh:Low (0.5 ppt) treatment tank, there was a significant difference between percent of time spent on either side of the tank, with a preference towards the low salinity side over the fresh water. In the Fresh:Medium (6.00 ppt) treatment tank, there was no significant difference in time spent on either side of the tank, between the populations, or averaged across both populations. This

pattern was also seen in the Fresh:High (12.00 ppt) treatment tank, with no significant difference in time spent on either tank side, tank side by population, or averaged across populations. The Medium:High (6.00 ppt, 12.00 ppt) also saw a significant difference between time spent on either side of the tanks, with a preference towards medium salinity water within the tank. None of the tanks above saw any significant difference in percent of time spent on either side of the tank between the two populations, as well as averaged between the two populations.

This pattern was not replicated when analyzing the difference in time spent in the differing water treatments. In the Fresh:Low treatment, there was a significant difference in time spent between the two water bodies, with more time spent in the low salinity water over the fresh water. This treatment also saw a significant difference in time between the Middle Area and Low salinity in both the Muddy Valley and Reynolds Creek populations. This was also seen in the Fresh:Medium treatment with a strong preference towards the fresh water in Muddy Valley newts and a slight preference towards fresh water in the Reynolds Creek newts. In Muddy Valley newts, there was a significant difference in time spent between the fresh water and the medium salinity water. Reynolds Creek newts in the Fresh:Medium salinity exhibited a strong difference between the fresh water and the middle area, as well as the middle area and the medium salinity water. In the Fresh:High treatment, there was a significant difference in time spent on either

side of the tank, with a strong preference for the fresh water over the high salinity water. Muddy Valley newts displayed a significant difference in time spent between the fresh water and the middle area, as well as the fresh water and the high salinity water, but did not show any significant differences between time spent on the middle area and the high salinity water. Reynolds Creek newts exhibited a significant difference time between the fresh water and middle area, as well as between the middle area and high salinity water, but none between the fresh water and the salt water. In the Medium:High treatment tank, there was a significant difference in time spent on either side of the tank, with a marginal favor towards the high salinity water in Muddy Valley newts, and a preference for medium salinity water in Reynolds Creek newts. Muddy Valley newts showed a significant difference in time spent in the middle area and the high salinity water, but in no other combination of areas. Reynolds Creek newts in the Medium:High treatment displayed a significant difference between time spent in the fresh water and the middle area, but no other combination of these factors.

In treatment tanks of lower salinity (0.5 ppt), both populations of newts displayed a preference to the slightly saline water. As salinity rose in the treatment tank, newts from the same populations began to exhibit different behaviors, with some favoring higher salinity, while others opted for the lower salinity water that was available. Reynolds Creek newts non-significantly preferred the medium salinity water over the fresh water, while the inverse was true for Muddy Valley newts

while other populations were in the Fresh:Medium treatment tank. In the Fresh:High tank, both populations marginally favored the fresh water over the high salinity water that was available. In the Medium:High treatment tank, neither population significantly favored the water body over the other, though there may be a slight, non-significant preference for the medium salinity water.

The combination of these factors indicates that there may be a conscious threshold or state of comfort that these newts may be experiencing in day to day life. Both populations favoring the low salinity over the completely freshwater indicates that there may be a naturally occurring requirement for some form of minerals or salinity to be present in the water to maintain their proper internal functions. When salinity rose to 6.00 ppt in the Fresh:Medium tank, a small preference towards the saline water by Reynolds Creek newts, but a preference towards the fresh water for the Muddy Valley newts suggests that salinities near or around that measurement may exceed or nearly exceed the normally encountered salinity for Muddy Valley newts, but not Reynolds Creek newts. In the Fresh:High treatment tank, both populations appeared to favor the fresh water in varying degrees of significance, indicating that somewhere between 6.00 ppt and 12.00 ppt may be the comfort or osmotic threshold for Reynolds Creek newts, who experience daily salinities higher than their counterparts in Muddy Valley. Both populations also appear to subtly favor the medium salinity water over the high salinity water in the Medium:High treatment tank, which further corroborates the above information.

Due to the small sample sizes and narrow availability of differing salinities, narrowing down an exact point of tolerance to prefer or avoid salinities is not possible from this data set. In order to gain more perspective and insight, running experimental tanks with more newts, more populations, and more differing salinity levels would be necessary to create a more robust collection of patterns and behaviors. The above data, however, does indicate that there may be some level of preference or behavior based on a threshold or tolerance level that may differ between populations. A conscious threshold of between 6.00 ppt and 12.00 ppt would loosely align with prior research that assessed newts from a freshwater environment having an upper salinity limit of roughly 11.00 ppt (Albecker et al., unpublished data). Additionally, strong behavioral preferences may not have been shown toward experimental treatments reaching 12.00 ppt if newts in some populations are regularly encountering these values and much more. If newts can travel to and withstand salinities over 25 ppt on a daily basis, the value of the experimental treatment tanks may not have been high enough to evoke a strong behavioral response from either population. Population location may play a role in the behavior of newts and their water preferences (Vegso et al., 2022), but a statistically insignificant difference between time spent on tank sides and treatment types across both populations indicated that there may be a genetic factor at play, as seen in other amphibian species, such as the coastal and inland populations of *Bufo japonica* in Japan (Haramura et al. 2019).

Conclusion

As stochasticity in aquatic environments is becoming more prevalent due to climate change, it is important to understand the surrounding factors that are shaping the way ecosystems form and behave. Many environmental stressors are increasing in frequency and intensity, and salinity is no exception. This naturally occurring compound can cause large-scale catastrophes to sensitive species like amphibians, who are vulnerable to contact with their skin. The increase in salinity can have detrimental effects not only for the habitats in which amphibians live, but also their breeding, oviposition, development, feeding, and other daily behaviors.

A lack of information on amphibian responses to salinity in field settings and the characteristics of the habitats that they are living in create a disconnect between the laboratory and the management plans that are implemented in hopes of conserving critical species and habitats. Even less is known about the behavior of amphibians in sites with introduced or encroaching salinity. This gap leaves a large amount of knowledge about the limitations of salinity tolerance within species as a mystery.

The behavior exhibited by newts in both controlled and unpredictable environments highlights the need to continually develop a more accurate understanding of salinity tolerance and behaviors in not just rough-skinned newts,

but potentially other species as well. The setting of the laboratory and the field are so drastically different that manufactured salinity and results from controlled experiments are suitable guidelines and starting points, but are far from the conditions and behaviors that they exhibit in their natural state.

As information is shared about field locations and amphibian presence in a wide variety of habitats, reevaluating the way that these ecosystems are labelled and classified is a must to continue to develop a more nuanced understanding of how susceptible species like the rough-skinned newt tolerate and navigate the uniquely complex waterbody that they live in and return to annually contribute to the population. Monitoring more populations of rough-skinned newts is vital to create a robust documentation of their habits and physiological responses to unfavorable environments. As an amphibian with a fair amount of published salinity tolerance(s), using it as a baseline for many other species can allow us to detect patterns across populations. The expansion of monitoring to amphibian species of conservation concern may allow for more practical and synergistic management plans and action from agencies and stakeholders alike.

With the climate becoming increasingly extreme and shaping the habitats of our wildlife, it is critical that long-term research and documentation of amphibian behaviors continue to properly comprehend their life cycle and flexibility in dynamic environments. Many coastal communities and their natural habitats are

undergoing large amounts of geographic change as storms continue to increase and sea levels begin to rise. This culmination of data builds the foundation for comprehending the dynamic changes in coastally located amphibian habitats and how the animals of these environments can exhibit flexibility and resilience, and continue to thrive despite their harrowing surroundings.

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