FOOD WASHING IN CAPTIVE NORTH AMERICAN RIVER OTTERS

(*LONTRA CANADENSIS*)

by

EVELYN NEUNTEUFEL

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

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Evelyn Neunteufel

Adviser: Dr. Richard Veit

Descriptions of food-washing in non-human animals are rare and often misinterpreted. For example, the commonly described "food-washing" by raccoons has been experimentally shown to be unrelated to cleansing and may instead be an artifact of captivity. I observed a captive North American river otter seeming to wash his food and investigated whether captive otters (1) washed food to remove sand; (2) carried food to water to specifically remove a loose substance from food or to also remove an outer shell; and (3) associated washing with a specific location (their pool), or would wash food at another location (a tub near the pool). I found that the otters (1) used water to wash sandy food more often than clean food; (2) did not carry food to water for manipulations when removing it from artificial toys and from natural shells; and (3) one of two otters washed in the tub when food was offered closer to the tub, but otherwise preferred to wash food in his pool. Other behaviors, such as swishing food, dropping food in water and retrieving it, pushing it through water, rolling with it in water, and rubbing it against rocks may

have aided in food cleansing. In contrast to published results from testing individuals of other species, captive otters truly wash their food.

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Romulus and Remus, but also remembered the needs of the researcher, who showed up day after day after day.

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Romulus

Table of Contents

List of Tables

List of Figures

1.0 Introduction

Animals are adaptively specialized to respond to functionally relevant properties of their habitats, that are necessary for their survival (Brown and Gass 1993; Shettleworth 1998; Hauser 2000). It is subsequently often assumed that animals form internal representations of the outside world (Cheney and Seyfarth 1990; Spaulding and Hauser 2005) to solve problems, though simpler processes can often explain apparently complex behaviors (Wehner 1997; Shettleworth 2001). For example, wood lice (*Oniscus asellus*) gather in humid areas to avoid dessication by simply increasing their activity in dry areas, until they accidentally enter a moist area where their activity slows down (Edney 1954).

The types of features that animals attend (such as color, shape or material) may be constrained by the physical environment, body size, or computational capabilities (Wehner 1997). Appropriately functional behaviors in response to certain features must usually be learned, and such ability to learn seems to reflect the evolutionary adaptations of the species. For example, Brown and Gass (1993) showed that hummingbirds (*Selasphorus rufus*) learned spatial association unusually rapidly and proposed that ecological and physiological characteristics of the birds caused this ability. Povinelli (2000) speculates that primates have an evolutionary predisposition to learn to manipulate objects. Inexperienced tamarins (*Saguinus oedipus*) and marmosets (*Callithrix jacchus*) are unable to discriminate between functionally relevant and irrelevant properties of a tool, but after little experience marmosets outperform tamarins in tool use (Spaulding and Hauser 2005). The authors explained that the marmoset's

superior performance may be due to a foraging pattern that requires a longer attention span than the foraging pattern of tamarins.

I inadvertently discovered washing behavior after videotaping North American river otters at a zoo. I had offered two otters dead capelin in their outdoor exhibit on several occasions. One day I observed an otter named Remus drop a fish onto the sandy ground. Instead of immediately consuming the fish, he picked it up and carried it to the pool. There he seemed to wash the fish, submerging and swishing it in the water, before he ate it.

Food-washing in non-human animals is seldom described in the literature, and some behaviors described as "food-washing" were later found to be something else. Japanese macaques (*Macaca fuscata*) living in semi-natural conditions wash potatoes and other food items (Kawai 1965; Scheurer and Thierry 1985; Nakamichi, Kato et al. 1997). Tufted capuchin monkeys (*Cebus apella*) and crabeating macaques (*Macaca fascicularis*) wash sandy food in captivity (Visalberghi and Fragaszy 1990), and some captive wedge-capped capuchins (*Cebus olivaceus*) wash food (Urbani 2001). Captive raccoons (*Procyon lotor*) so frequently dip food items into water, that a number of researchers studied this behavior in detail (MacClintock 1981). A husbandry manual for the Asian small clawed otter (*Aonyx cinerea*) states that they "have been observed to wash their food" (Lombardi and O'Connor 1998). Wild European river otters (*Lutra lutra*) sometimes rub eels on the ground and then take them back to the water (Kruuk 1995). Kruuk speculated that this behavior functioned in removing slime from eels, though he

did not use the term “washing.” Though over 25 bird species dunk food in the wild, only half are described as “washing” their food (Morand-Ferron, Lefebvre et al. 2004).

Previous authors have used the term “washing” without defining it. They have also described the behavior as dunking (Wible 1975), swishing (Wible 1975), submerging food and moving it from side to side (Johnson 1976; Seibt and Wickler 1978), dipping and rubbing (Kawai 1965; Scheurer and Thierry 1985; Urbani 2001), dropping (Kawai 1965; Pitochelli 1985) food in water, and holding food under running water (Pitochelli 1985). Webster’s Third New International Dictionary of the English Language defines “wash” as “to cleanse by the action of water or other liquid: dip, rub, or scrub in or with a liquid for the purpose of cleansing.” Gewalt (1956) defined it as “ein Abreiben und Abspülen von Schmutz unter Wasserbenutzung”, which means to use water to rub and rinse off dirt. Visalberghi and Fragaszy (1990) interpreted the behavior of monkeys as “unambiguous washing” when eating clean food was not alternated with eating sandy food and when washing was alternated with visual inspection until most of the sand was removed before the food was eaten.

Often washing behaviors have been interpreted as demonstrating “insight”, as by Seibt and Wickler (1978), who describe dung beetle washing by maribou storks, and by Pitochelli (1985) concerning food washing by wild grackles. Sometimes washing is regarded as “innovative” or “cognitively sophisticated” (Morand-Ferron, Lefebvre et al. 2004), requiring the actor to relate food and water to one another. In many of the above-

mentioned cases, a cleansing function of the behavior seems to have been assumed, but neither the function nor the development of the behavior has been tested.

Food washing by primates has often been presented as an example of a cultural phenomenon (Tateishi and U. 1958; Kawai 1965; Scheurer and Thierry 1985; Nakamichi, Kato et al. 1997), that is, discovered by one individual and then spread by imitation, such as sweet-potato washing (Kawai 1965) by Japanese macaques (*Macaca fuscata*). Grass roots washing, sometimes accompanied by rubbing the roots along a rock at the edge of a river, spread in a similar way (Nakamichi, Kato et al. 1997). However, Urbani (2001) observed the washing behavior of four wedge-capped capuchin monkeys (*Cebus olivaceus*) without noticing any indication of transmission of the behavior from one monkey to another. Washing may be spread and maintained by trial and error learning (Galef 1992; Tomasello and Call 1997), rather than imitation.

Detailed experiments analyzing the dunking of food into water have been performed with raccoons (Gewalt 1956; Lyall-Watson 1963), crabeating macaques (*Macaca fascicularis*) and capuchin monkeys (*Cebus apella*) in captivity (Visalberghi and Fragaszy 1990), and with Carib grackles (*Quiscalus lugubris*) in captivity and in the field (Morand-Ferron, Lefebvre et al. 2004). Research to determine the function and extent of such behaviors among species may prove useful for further understanding the diverse cognitive abilities of animal species.

For my experiments, I defined washing as "the use of water to remove substances from the surface of objects." Assuming that Kruuk's interpretation that the otters mentioned above rubbed and doused eels for the purpose of removing slime was correct, then I call this behavior "washing."

The questions I addressed were: 1.) Will captive otters wash their food? 2.) Will otters use water adaptively, to wash off loose substances such as sand, but not substances such as shells, which cannot be washed off? 3.) Will otters associate a certain location (their regular pool) with clean food?

I spent several months observing the regular activities of two otters in their outdoor enclosure, noting the types of object manipulations performed in water and on land. Then I tried several experimental methods, to determine which would be possible in a zoo environment. I designed a series of experiments to test the ideas outlined above at Essex County Turtle Back Zoo (West Orange, NJ) and at four other zoos, chosen to approximate the conditions at Turtle Back Zoo and to allow for separation of the otters for individual testing. The objectives and rationale for the experiments were as follows:

Experiment 1: Fish Dipped in Sand vs. Clean Fish

Objective (questions and hypothesis): The objective was to determine whether captive North American river otters remove sand from the surface of food items. Do otters wash sandy fish before eating them?

Hypothesis: Otters will wash sand off a fish before eating it.

Experiment 2: Fish in Toys vs. Free Fish

Objective (questions and hypothesis): The objective was to determine whether otters use water appropriately to wash (remove loose substances) from the outside of food. Food objects are often covered with sand or other grit, which can easily be removed by washing. Alternatively, objects can be encased in a shell or net of interconnected material that cannot be washed off. Examples of the latter in nature are the housings of crabs or mollusks. These substances have to be removed from the outside of the edible part of the food item in some other way, such as by crushing or by forcefully pulling out the edible morsel from its shell.

Do otters understand that, though swishing a fish in water can remove small pieces of loose substance such as sand from a food item, it cannot remove an item such as a "cage-like" rubber dog toy (into which the food has been inserted)? Will the otter attempt to wash the toy off? I want to determine whether an otter will carry a food item to water, if an inedible (or undesirable) substance is present on the surface of the food, whether water itself directly aids in removing this substance or not.

Hypothesis: Otters will carry fish requiring manipulation (to remove undesirable material) to water, even if water does not directly aid in removing this undesirable material (i.e. rubber dog toy).

Experiment 3: Handling of Sandy Food With and Without Shells and Clean Food With and Without Shells

Objective (questions and hypothesis): The objective was to determine if other captive otters remove sand from food items by carrying them to water and swishing them. I also wanted to determine if the otters carry food to water to remove a natural outer food covering, such as a natural mussel shell.

Hypothesis: Captive otters wash sand off food before eating it. Otters will also carry a food item requiring manipulation (to remove undesirable material) to water, even if water does not directly aid in removing this undesirable material (i.e. mussel shells).

Experiments 4 and 5: Availability of Alternate Source of Water

Objective (questions and hypothesis): I wanted to test whether otters are able to conceptualize washing. If they can, then they ought to carry food to sources of water other than the pool to wash it. To test this, I placed a tub filled with water near the pool. They should use the tub whenever the food is located closer to the tub than to the pool, since carrying the food a shorter distance to water is more efficient. They should use the

pool and tub equally often if the food is equidistant from the tub and the pool. They should use the pool when the food is placed closer to the pool than to the tub. The key feature is the water itself, not the location of the water.

Hypothesis: Otters wash food in the closest available source of water.

1.1 Background

1.1.1 Cognitive Abilities in Animals

In a set of several experiments to test the recognition of relevant features for tool use (Hauser, Pearson et al. 2002), infant cottontop tamarins (*Saguinus oedipus*) learned to use a hard blue cane to retrieve food. When the monkeys were later offered a choice of tools with different properties, they disregarded differences in color and texture, but attended to size and shape, demonstrating that they understood which feature categories are relevant for a tool's usefulness. In another set of experiments (Spaulding and Hauser 2005), naïve tamarins (*Saguinus oedipus*) and marmosets (*Callithrix jacchus*) chose between tools with different features affecting functionality, such as a clay cane (functional) versus a cane shaped rope that would unravel (non-functional), to pull a food item towards them. After several trials, marmosets learned to pick the functional tool, but tamarins did not. However a "tool" made of disconnected wood chips (laid out in a tool-like shape) was recognized as non-functional by both species. These experiments

demonstrate the differential abilities of species to attend to relevant properties of objects in their environments.

Animals can respond with different levels of cognitive complexity. The complexity of object manipulations by various non-human primates is frequently compared to that of human infants (Parker and Gibson 1977; Tomasello and Call 1997). Parker and Gibson (1977) described five categories of object manipulation, adding that these categories say nothing about the flexibility of the behaviors:

> (1) Simple prehension -- grasping an object in one or both hands (or in other anatomical equipment such as mouth and/or claws, trunk, etc.).
> (2) Simple object manipulation -- coordinated manipulation of one object with one or two manipulators, or sequential manipulation of two objects with one or two manipulators, e.g. object transferring from hand to hand, object rotation, object uncovering followed by grasping.
> (3) Object-substrate manipulation-- manipulation of one object (with one or two manipulators) relative to a fixed substrate or medium, e.g. rubbing an object on the ground, or washing an object in water, or banging an object on a surface, or throwing an object onto a surface.
> (4) Complex object manipulation-- manipulation of one detached object relative to another (and in some cases through a force field) involving subsequent change of state of one or both of the objects, e.g. hitting one object with another, either

directly or by throwing, raking in one object with another, opening one object with another as a lever.

(5) Social-object manipulation--manipulation of an object relative to other animals without direct physical contact between the object and other animals, e.g. baiting another animal with food, agonistic displays with objects, resulting in changed behavior in the receiver.

Recent studies have shown that the demonstration of learned behaviors is affected by social and other environmental conditions. The presence of conspecifics can either facilitate (Moscovice and Snowdon 2006) or inhibit learned behaviors (Drea and Wallen 1999; Schaik, Deaner et al. 1999). In several cases, stealing by conspecifics inhibited food handling (Visalberghi and Fragaszy 1990; Bugnyar and Kotrschal 2002; Morand-Ferron, Lefebvre et al. 2004; Morand-Ferron, Veillette et al. 2006). Social rank influences the demonstration of learned behaviors, with higher ranking starlings (*Sturnus vulgaris*) outperforming lower ranking companions (Boogert, Reader et al. 2006) and subordinate monkeys (*Macaca mulatta*) performing better, when tested without the presence of dominant monkeys (Drea and Wallen 1999). Due to a higher prevalence of food dunking by individually tested captive grackles than by wild grackles, Morand-Ferron (2006) speculated that stealing reduced the prevalence of food dunking in wild grackles. Other studies also found behaviors present in captive animals, which were not or rarely seen in the wild (Schaik, Deaner et al. 1999; Tebbich and Bshary 2004; Breuer, Ndoundou-Hockemba et al. 2005).

1.1.2 Some Mammals and Birds Wash Their Food

The raccoon is called „raton laveur“ (French), meaning „little washing rat“, “Waschbär“ (German) and „oso lavador“ (Spanish), both meaning „washing bear“, and *Procyon lotor* (Latin), meaning „pre-dog washer.“ Understandably, several researchers have studied its „washing“ behavior. Raccoons carry objects to water, douse, rub and drop them in the water, and then dabble, by paddling with their hands in the water, while fingers are spread, as if searching for food (Lyall-Watson 1963; MacClintock 1981; Zeveloff 2002). They may wash food and non-food items (Gewalt 1956; Lyall-Watson 1963). Though the dousing and rubbing of objects is superficially similar to washing, most researchers agree that this behavior is not for the purpose of cleaning (Lydekker 1894; Bierens De Haan 1932; Gewalt 1956; Lyall-Watson 1963). Lyall-Watson (1963), for example, showed that captive raccoons did not douse muddy crabs more often than clean crabs. An alternative explanation for the raccoon's dousing behavior is a need to moisten food before eating it (Lydekker 1894). However, in contrast to earlier beliefs, raccoons have “well developed” salivary glands (Neseni 1938; MacClintock 1981) and they will not douse dry food more often than wet food (Gewalt 1956; Lyall-Watson 1963). Bierens De Haan (1932) suggested that washing was a form of play without any other function. Gewalt (1956) proposed that raccoons show a propensity to dabble and incapacitate prey with their paws before eating, to avoid touching potentially dangerous prey with their mouths. However, he did not explain their need to do this in water. Since wild raccoons search for food by dabbling in muddy waters with their front paws, Lyall-Watson (1963) speculated that captive raccoons wash objects due to a natural propensity

for behavior patterns, which are typical for foraging in the wild. None of these three alternative explanations were tested.

Captive crabeating macaques (*Macaca fascicularis*) and capuchin monkeys (*Cebus apella*) both begin to wash sandy food, when it is offered near a source of water (Visalberghi and Fragaszy 1990). Food is dunked, rubbed against a wall in the water, dropped and retrieved. Although crabeating macaques, which naturally forage in water, might be expected to show a greater tendency to wash food than capuchins, which rarely approach water in the wild, in captivity both species learn to wash food through individual experience. However, Visalberghi and Fragaszy concluded that washing was sometimes inappropriate, such as when the capuchins caught and doused a (presumably clean) lizard.

Free-ranging Japanese macaques (*Macaca fuscata*) also wash by dunking and sometimes rubbing food along a hard (rock) surface (Nakamichi, Kato et al. 1997). Some captive Japanese macaques wash, by pressing a water spout and rubbing food with their hands under the stream of water (Scheurer and Thierry 1985). Since macaques with improved performance in sand-digging tests learn faster to wash food (Tsumori, Kawai et al. 1965), Scheurer and Thierry (1985) suggest that behavior patterns used for washing may have developed from foraging behaviors, such as digging.

Carib grackles (*Quiscalus lugubris*) wash by repeatedly dropping and retrieving food in water. Wild grackles wash clean bread as often as sandy bread, but dry bread more often

than moist bread, showing that they dunk food for moistening, not for cleaning (Morand-Ferron, Lefebvre et al. 2004). More captive grackles (86%) dunk food than wild grackles (61%). Social factors, such as high bird density associated with increased stealing, decrease the likelihood of dunking (Morand-Ferron, Lefebvre et al. 2004; Morand-Ferron, Veillette et al. 2006).

Washing foods to remove unpalatable, toxic or indigestible substances should have clear fitness implications for animals. Yet, washing is relatively rare. Research to determine the prevalence and function of washing among species may be useful to improve our knowledge of the diverse cognitive abilities of species. To date, washing has not been studied with otters.

1.1.3 Otter Behavior

The six otter genera belong to the weasel family, Mustelidae, (Feldhamer, Drickamer et al. 1999; Nowak 1999). Whereas sea otters (*Enhydra lutris*) are marine mammals (VanBlaricom and Estes 1987), river otters are semi-aquatic (Kruuk 1995; Dunstone 1998; Williams, Ben-David et al. 2002). Otters are grouped into two foraging types (Chanin 1985; Dunstone 1998), the fish specialists (*Lontra, Lutra, Pteronura*) and the invertebrate specialists (*Aonyx, Enhydra*). North American river otters (*Lontra canadensis*) live in rivers, lakes, freshwater marshes, salt marshes and sea coasts (Melquist and Hornocker 1983; Polechla 1990; Newman and Griffin 1994; Ben-David, Bowyer et al. 1996) and forage mostly in water (Beckel 1990; Serfass 1995; Blundell,

Ben-David et al. 2002), consuming prey such as brook stickleback (*Culaea inconstans*), northern pike (*Esox lucius*), white sucker (*Catostomous commersoni*), salmon (*Salmo salar*), crayfish (*Procambarus sp.*), blue crabs (*Callinectes sapidus*), shrimp, snapping turtles (*Chelydra serpentina*), beavers (*Castor canadensis*), waterfowl, and shellfish (Chabreck 1982; Gilbert and Nancekivell 1982; Melquist and Hornocker 1983; Stenson, Badgero et al. 1984; Carss, Kruuk et al. 1990; Brooks, Brown et al. 1991; Reid, Code et al. 1994; Serfass 1995; Erlandson and Moss 2001). Most often, otters dive straight down in shallow water to catch immobile or slow-moving prey (Chanin 1985; Kruuk 1995; Dunstone 1998). They may mouth food, roll around with it in the water, release and retrieve it (Kruuk 1995). Small prey is usually eaten at the water surface, but large prey is consumed on land (Chanin 1985; Stokes and Stokes 1986; Kruuk 1995). The river otters, especially the genera *Lontra*, *Lutra* and *Pteronura*, forage by similar means (Chanin 1985; VanBlaricom and Estes 1987; Kruuk 1995), though Asian small-clawed otters (*Aonyx cinerea*) are more prone to catch prey with their paws (Lombardi and O'Connor 1998).

The otter's high energy needs (Kruuk, Balharry et al. 1994) require efficient foraging strategies with a thorough knowledge of a variety of differentially profitable foraging sites (Carss 1995; Kruuk 1995). Otters (*Lutra lutra*) readily adjust to seasonal prey availability, exploiting migrating salmon (Carss, Kruuk et al. 1990), and switching from coastal to freshwater prey (Clavero, Prenda et al. 2006), though human disturbance may also be a factor (Carss 1995). Otters (*Lutra lutra*) easily switched to a novel prey species after the appearance of introduced American crayfish (*Procambarus clarkii*) in

Portuguese streams (Beja 1996). Developmentally, otter cubs usually remain with their mother for about a year, longer than most carnivore cubs (Stokes and Stokes 1986; Carss 1995; Kruuk 1995). Cubs begin to capture prey at about five months and foraging efficiency slowly increases, with sub-adults still capturing more of the less profitable prey than adults (crustaceans vs. fish), implying a long learning period (Watt 1993; Polotti, Prigioni et al. 1995). The otter's flexibility in diet and habitat use suggest complex foraging strategies (Carss 1995), which have to be learned. I would expect wild otters to show great flexibility in their use of water as a resource for food acquisition as described for European river otters by Kruuk (1995). Coastal river otters should exploit aquatic food resources in locations varying with the changing tides Kruuk (1995), while otters in freshwater habitats may be required to adjust the location of their foraging sites according to flooding after heavy rains and drying up of known water resources during droughts.

Captive otters might carry food items to water to wash them. As hypothesized for macaques (Scheurer and Thierry 1985), the development of this behavior might be due to the particular captive environment in which the animal lives (an enclosure containing sand in proximity to water) and/or to a natural tendency of the species to wash food. An alternative explanation might be that, in captivity, washing replaces natural foraging behaviors. Unable to hunt live prey, captive otters may carry food items to a pool of water to satisfy a natural propensity to manipulate food in water. If otters wash their food, flexibility in their use of sites for washing should reflect flexibility in their use of foraging sites. However, low motivation to handle food by well-fed captive animals, such

as the otters in my studies, may decrease generalization to novel stimuli (Shettleworth 1998).

2.0 General Methods

2.1 Subjects and Environments

The subjects for all tests were adult North American river otters held in captivity at zoos in New Jersey, New York and Connecticut (Table 2.1). The shell study (experiment 3) included seven otters at four zoos, whereas the subjects for all previous experiments and the tub studies (experiments 4 and 5) were two adult male otters at Essex County Turtle Back Zoo. An additional test with two male otters was begun at Beardsley Zoo in Connecticut, but discontinued. The otters in the shell study had diverse histories. The current environments of otters in the shell study varied from a completely enclosed indoor exhibit with artificial rock and glass windows at the Maritime Aquarium, to a fenced-in temperate forest with a dammed stream at Trevor Zoo in Millbrook, New York. Therefore the amount of experience with sand and types of food items is likely to have differed between otters. Based on information I obtained from the respective zoos, it is unlikely that any of the otters had been trained to wash food.

Table 2.1 Otter Histories and Exhibits

Facility	**Otter Name**	**Sex**	**Place of Birth (for Wild Born) and Age at confinement**	**Otter's Age at Testing (years)**	**Exhibit**
Essex County Turtle Back Zoo (TBZ) West Orange, NJ	Romulus	M	captive-born	6-8	sand, earth, stones, rocks, vegetation, artificial pool
Essex County Turtle Back Zoo (TBZ) West Orange, NJ	Remus	M	captive-born	6-8	sand, earth, stones, rocks, vegetation, artificial pool
The Maritime Aquarium (MAQ) Norwalk, CT	Bell	F	captive-born	10	artificial rock, artificial pool
Maritime Aquarium (MAQ) Norwalk, CT	Lew	M	Florida < 1 year old	2	artificial rock, artificial pool
Trevor Zoo (TZ) Millbrook, NY	Otto	M	Louisiana < 5 years old	9	sand, earth, stones, rocks, vegetation, natural stream-fed pond
Trevor Zoo (TZ) Millbrook, NY	April	F	South Carolina < 2 months old	3	sand, earth, stones, rocks, vegetation, natural stream-fed pond
Trailside Zoo (BMZ) Bear Mountain State Park, NY	Dell	M	New York ? < 6 months old	10	artificial rock, artificial pool
Connecticut's Beardsley Zoo (BZ) Bridgeport, CT	Chewy	M	captive-born	18	earth, stones, rocks, sod, vegetation, artificial pool
Connecticut's Beardsley Zoo (BZ) Bridgeport, CT	Rizzo	M	captive-born	2	earth, stones, rocks, sod, vegetation, artificial pool

2.2 Materials and Methods

The food I used for the experiments was either chosen from foods that the otters regularly ate, or tested for palatability before starting the experiments. The food was stored frozen and then defrosted the night before, or the morning of, the testing. Capelin (*Mallotus villosus*), a slender small-scaled species of smelt (family Osmeridae), was used for most experiments. Shortly before the start of experiment 2, capelin became unavailable and trout (*Salmo gairdneri*) was used throughout this test. An attempt to use fishballs for testing was soon abandoned, because otters had difficulty removing fishballs from shells, the fishballs broke apart during manipulations, and fishball palatability was low.

I chose the size of food items so that no item offered would be rejected on this basis. Food items needed to be large enough for otters to manipulate and eat, in spite of handling costs (such as washing off sand). Yet they needed to be small enough for the otters to eat all the food items offered without becoming satiated after eating the first piece(s). This determination was often difficult, as feeding of otters by regular zoo staff was not always consistent between and even within zoos. Some zoos kept poor records on amounts fed, and I found it difficult to ascertain how much the otters had eaten. For otters in zoos with outdoor enclosures, I expected the fluctuation of environmental temperatures to affect appetite. In addition, the otters at the Trevor Zoo were able to supplement their diet with live food caught in their own enclosure. I kept the size of the food items that I presented consistent throughout each experiment.

All otters were tested in the morning, usually between 9 AM and 11AM. The otters were locked up so that they were unable to see the preparation or distribution of food items. I wore natural rubber latex gloves during preparation. I dipped food items for the "sandy" treatment into fine sand (commercially available non-toxic and uncolored "marine sand" for use in aquariums), placing the food onto the sand in a plastic container and pressing it down so that the sand stuck to the food items. For some treatments (see methods for individual experiments), I inserted the food items into objects, such as plastic toys or scraped out mussel shells.

Test items were distributed in the test area near the pool in the otters' regular enclosure. No other source of water was available in the enclosure. (At some zoos the pool itself was fed by a waterfall or a pipe with running water, but the otter would have had to enter the pool to access this source of running water.) All humans left the otter exhibit before an otter was released for testing, and remained out of view of the otter being tested. At Turtle Back Zoo and Trevor Zoo, I hid behind walls and vegetation. At the Maritime Aquarium vision was poor from the lit otter exhibit into the dark hallway, where I was stationed at the camcorder. Due to lack of hiding places at the Trailside Museums and Zoo, I manufactured and hid behind a cardboard blind. Testing at Turtle Back Zoo, the Trailside Museums and Zoo, and the Maritime Aquarium was usually done before the facility was opened to the public, though sometimes testing was delayed due to unforeseen circumstances and some visitors appeared before testing was finished. Trevor Zoo was opened to the public when keepers arrived for work, but usually the day's

testing was completed before any visitors arrived. Any visitors were politely asked to quietly stand a few feet back from the exhibit.

I videotaped the otters' behaviors in the vicinity of the food set-up using a Sony DCR-TRV50 digital video camera recorder using mini DV cassettes. Prior to the start of testing, each exhibit was videotaped from various angles to record the layout of the exhibit and to determine optimal requirements for videotaping. During testing, the camcorder was always positioned with the pool between camcorder and test area. In order not to influence the otters' behaviors, I remained out of view of the otters during testing.

2.2.1 Analysis and Archiving of Videotapes

Videotapes were labeled by date, but there was no mention on the tapes or tape labels of the test treatment used on that day. For analysis, the videotapes were viewed on a television screen or computer monitor and the data recorded on Excel sheets. For precise data recording, portions of all videos were watched at slow motion or even frame-by-frame. Many scenes were viewed several times as necessary to determine exactly when certain behaviors occurred (such as when the food was lifted by the otter or when the food touched the water), whether food was swished or possibly just shaken above the water surface, and how it was otherwise manipulated.

3.0 Experiment 1: Washing of Fish Dipped in Sand vs. Clean Fish

I predicted that sandy fish would be carried to water and submerged. Though even slight movements through the water without vigorous swishing could remove sand from the fish, swishing the fish was taken as clear evidence of washing. On any day, two otters were offered either clean or sandy capelin, with the order of the treatments randomized.

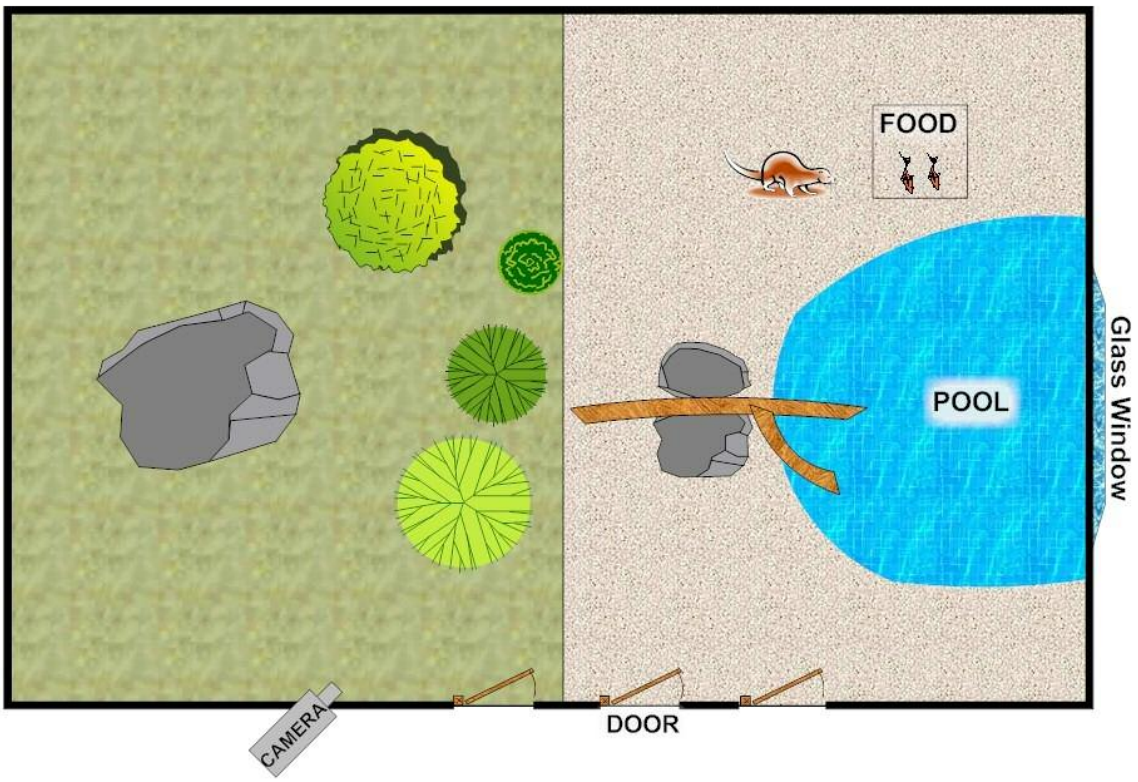
3.1 Materials and Methods

3.1.1 Subjects and Environment

The subjects were two adult male North American river otters named Romulus and Remus (Table 2.1) housed together in a naturalistic outdoor exhibit (Figures 3.1 and 3.2) for approximately 6 hours during the day and kept in indoor cages from late afternoon until the next morning. I suspected that Romulus was dominant over Remus for several reasons: 1) Romulus was usually the first to approach unfamiliar objects. 2) Romulus was usually the first to exit the door. 3) Romulus nipped Remus after Remus took a fish located directly in front of Romulus' nose.

Figure 3.1 View from Camcorder to Test Area at Turtle Back Zoo

Figure 3.2 Overhead Schematic of the Otter Exhibit at Turtle Back Zoo as for Experiments 1, 2, and 3. The exhibit contained a pool of filtered water. The bottom and edges of the pool consisted of cement and a glass underwater viewing window. The ground near the pool was rocky with some sand. The remainder of the exhibit contained rocks, pebbles, logs, grass, small trees and shrubs. The food for testing was placed in a bowl or on a rectangular piece of Plexiglas (50 cm from the pool). Otter drawing inserted for scale.



The indoor cages consisted of cement and metal grids. Each cage had a separate door to the outside exhibit. The otters were usually let out in the morning and allowed to return inside in the afternoon. Doors connecting the three indoor cages were left open at night, for the otters to move freely between the cages. One or two rubber tubs filled with water were available in one of the cages. There were no loose substrates (such sand, straw, earth, or wood chips) in the cages. The keepers washed the cages daily while the otters were outdoors.

The otters were offered food once a day in the late afternoon. Food was placed into clean metal bowls in the indoor cage farthest from the cage with the water tub. The otters were then allowed to come inside. Depending on the day, they were fed meatballs and chicken, or meatballs and fish (capelin or small trout). All food preparation and washing of bowls was done in a separate room, not visible to the otters. In addition to their daily feeding, the otters occasionally received defrosted fish (sometimes inserted into toys or frozen in chunks of ice) thrown into their outdoor pool for enrichment (behavioral stimulation).

3.1.2 Data Collection

I fed the otters capelin since they had been regularly eating this species. Capelin was shipped frozen in large cartons and defrosted by the zoo as needed. For experiments, these fish (18 cm) were taken from the zoo's frozen supply, defrosted and used for testing. Wearing natural rubber latex gloves, I prepared capelin for the "sandy" treatment by laying the fish into a dish containing fine aquarium sand and turning the fish to coat all sides with as much sand as would stick. "Clean" fish were pressed into a clean container (as if dipping them into sand). After treatment, I placed two clean fish or two sandy fish into a clean plastic bowl. All containers and bowls were washed, bleached and rinsed well after each use. The order of treatments was randomized.

The pool was cleaned of leaves, branches, and food before experimentation. I set the food bowl down on the sandy ground 50 cm from the pool, left the enclosure and released both

otters simultaneously into the enclosure. The otters remained in their outdoor enclosure for the remainder of the day.

The camcorder was set up behind the back wall of the otter exhibit to record otter activities on land and in the water near the food bowl. In order not to influence the otters' behavior, I remained out of view of the otters for the duration of the recording, other than to check for the presence of food every 15 minutes and to change videotapes after an hour, as necessary.

3.1.3 Analyses

I labeled videotapes and analyzed them as described in Section 2.2.1. I continued testing until at least 20 capelin per treatment had been consumed, discarding any trials in which both otters touched the same food item (i.e. capelin manipulated by one otter, then by the other). I recorded the name of the otter grabbing the food item and his subsequent actions following a predetermined coding of behaviors (Figure 3.3). I calculated Pearson's Chi Square with Yates correction for continuity. I used a simulation (with 2000 replicates) to find the p-values (R Development Core Team 2006) to compensate for empty cells.

Figure 3.3 Data Recorded for Experiment 1: Washing of Fish Dipped in Sand vs. Clean Fish

Time Lift fish = Time otter lifts fish out of bowl for first time.

Mouth or Paw = Body part used to lift fish out of bowl. (M = mouth, P = paw)

5secLocation = Location of fish 5 seconds after time noted in column "Time Lift Fish" with respect to location of fish in bowl

Water = Whether fish touches the water in the pool anytime before it is eaten. (Y = yes, N = no)

Time Touch Water = Time fish first comes in contact with water.

Time: Bowl-Water = Seconds elapsed between "Time Lift Fish" to "Time Touch Water"

Fish Touched Land = Whether the fish comes in contact with the ground anytime before it is eaten. (Y = yes, N = no)

Submerged Fish = If the fish is taken to water, whether it becomes completely submerged. (Y = yes, N = no)

Swished Fish = Whether the otter swishes the fish in the water (i.e. moves it back and forth with quick motions). (Y = yes, N = no)

Location Swallowed Fish = Location the fish is eaten.

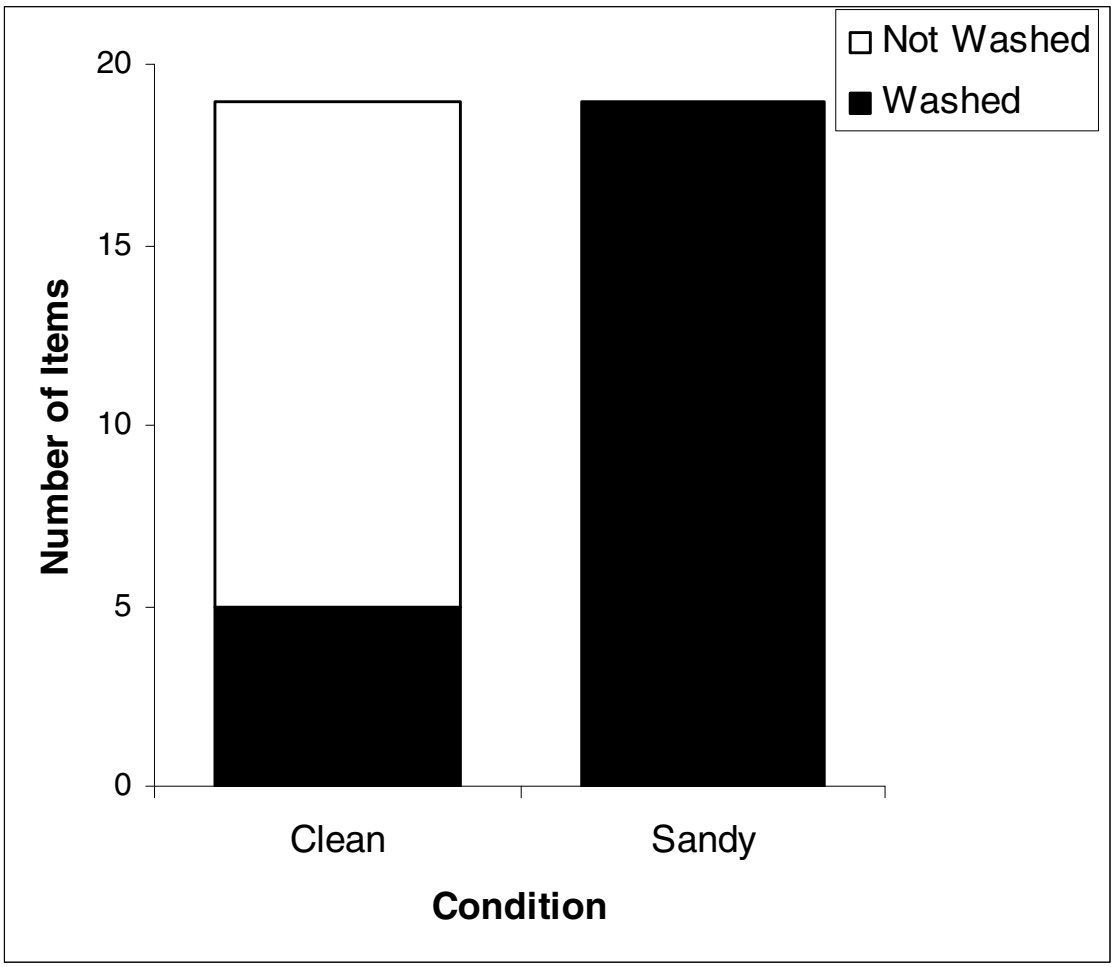
Other Otter lifted Fish Outside of Bowl = Once the fish had been lifted by one otter, did the second otter lift the fish at any time? (Y = yes, N = no)

3.2 Results

Experiment 1 (clean fish vs. sandy fish) required 22 days of testing from April 2003 to June 2003. Of 44 fish offered (20 clean fish, 24 sandy fish), Remus took 19 clean fish and 19 sandy fish out of the bowl and consumed them. Romulus consumed only 1 clean fish and 2 sandy fish. Romulus threw three additional sandy fish out of the bowl and dropped them onto the ground, but did not consume them.

Remus washed (submerged and swished) every sandy fish that he ate (Figure 3.4), including the three fish that Romulus had left lying next to the bowl (though these three fish were not counted in the analysis). Remus submerged and swished 5 clean fish (26%). Therefore Remus reliably washed sandy fish, but not clean fish (Yates Corrected X^2=19.113, d.f.=1, p<0.001). Of the 5 clean fish he took to the pool, 3 touched the ground before they were eaten, which could have caused sand to get on them and explain the subsequent washing. Romulus washed the 3 fish that he consumed.

Figure 3.4 Number of Food Items Washed by Remus



3.3 Discussion

These results indicate that Remus differentiated between clean and sandy fish, since he washed sandy fish more often than clean fish. Though even slight movements through the water without vigorous swishing could remove some sand from the fish, swishing the fish reinforced the notion that the otter was washing its food.

I wanted to test whether otters would wash materials other than sand off the fish (Table 3.1). To do this, I dipped each fish (trout) in the material, or the material (grass, seaweed) was wrapped around the fish. I offered each treated fish in a food bowl on two separate days (except fish wrapped in seaweed) with both otters present, and videotaped the otters' behavioral responses. Romulus washed some fish, but Remus did not try to wash any of the materials off the fish.

Table 3.1 Washing of Non-Sand Materials
In this table "washed" means taken to water. Seaweed was sushi nori. "Grass tied on" and "seaweed wrap" refer to substances wrapped and tied around the fish, so that they would be difficult to remove, even by swishing in water.

	1st Try-Response		2nd Try-Response	
Material	Romulus	Remus	Romulus	Remus
grass clippings	washed 2 fish	ate 2 fish w/grass	washed 3 fish	undetermined
grass tied on	ate 2 fish w/grass	ate 2 fish w/grass	washed 2 fish; ate1 (grass fell off)	ate 1 w/grass
chopped lettuce	did not eat	ate 2 fish w/lettuce	washed 1 fish	ate 1 fish w/lettuce
chopped seaweed	washed 1 fish	ate 1 fish w/seaweed	washed 1 fish	ate 1 fish w/seaweed
seaweed wrap	ate 3 fish w/seaweed	did not eat	not offered	not offered
boiled egg yolk	ate 2 fish w/yolk	licked yolk from empty bowl	ate 1 fish w/yolk	ate 1 fish w/yolk
bread crumbs	washed 1 fish	did not eat	washed 1 fish	ate 1 fish w/bread crumbs
uncooked rice	did not eat	ate 2 fish w/rice	washed 1 fish	ate 1 fish w/rice
tomato sauce	washed 3 fish, ate 1 w/sauce	did not eat	ate 1 with sauce, washed 1 piece of fish left by Remus	partly ate 1 fish w/sauce
orange juice	did not eat	ate 2 fish w/juice	ate 1 fish w/juice	ate 1 fish w/juice
honey	washed 1 fish	ate 1 fish w/honey	did not eat	ate 2 fish w/honey

Remus' enclosure, which contained a food source and sandy ground in proximity to water, was likely to have been conducive to the development of washing behavior. Visalberghi and Fragaszy (1990) supplied food in a container of sand in proximity to water, and the monkeys they studied readily learned to wash. This setting likely approximates habitats of wild otters. Whereas wild river otters seldom encounter prey on land (Stenson, Badgero et al. 1984; Reid, Code et al. 1994; Erlandson and Moss 2001), they consume large meals on shore (Stokes and Stokes 1986; Kruuk 1995), so their food may become

coated with sand or mud, which they need to remove. Possible disadvantages of such behavior in the wild are increased food handling time, losing the food to an aquatic competitor, and risks to the otter's own safety.

Romulus' lack of motivation to eat may have been due to several factors. I suspected that Romulus was the dominant otter, based on several observed behaviors, and he may have received more food during competition at the food bowls at night. He may have not been hungry enough to warrant expending extra energy for food handling. Remus usually carried sandy fish in his mouth, while Romulus seemed more reluctant to do so and instead tended to swipe fish out of the bowl using his paw, and pushed the fish towards the pool with his paw and nose, a much more time-consuming effort. Of the 6 fish he did touch, 5 happened to have been sandy and may have caused him to lose interest in approaching the food bowl thereafter.

None of the other materials with which I coated fish were washed off by Remus. Though Romulus washed 8 of the 11 materials off at least once, he did not consistently do so. It seemed that these substances were palatable enough for the otters to consume them with the fish and I did not believe that further study with these substances was warranted.

4.0 Experiment 2: Handling of Fish Dipped in Sand vs. Fish-in-Toys vs. Free Fish

The objective was to determine whether an otter will carry a food item to water, if an inedible (or undesirable) substance is present on the surface of the food, whether water itself directly aids in removing this substance or not.

I offered two otters clean trout, sandy trout, or trout inserted into toys on separate days, with the order of treatments randomized. I predicted that if the otter was predisposed to carry any food object requiring manipulation to water, he should carry sandy fish and fish within a toy to the pool for removing sand from the fish or fish from the toy. If, however, the otter used water only to wash off loose substances, then he should carry the sandy fish to the pool, but not the fish inside the toy. I expected the otter to eat the clean fish without approaching the pool.

4.1 Materials and Methods

4.1.1 Subjects and Environment

I used Romulus and Remus for this experiment. They were housed under the same conditions as during experiment 1. I used trout instead of capelin.

4.1.2 Data Collection

The otters received either four pieces of clean trout, four pieces of sandy trout, or four pieces of (clean) trout within toys each day. The order of the treatments (i.e. clean fish vs. sandy fish vs. fish-in-toys) was randomized. Since only one otter had consistently consumed test fish during the first experiment, I continued this second experiment until one otter ate at least twenty pieces of fish for each treatment (discarding any trials in which both otters touched the same food item). Even though this could result in the otter's eating more than twenty items from some treatments, all data were to be used for analysis.

Small trout (10-13cm) were used whole. I sliced the bodies of trout longer than 13cm in half lengthwise (approximately along the lateral line) after cutting off and discarding their heads. Clean trout (sliced or whole) and sandy trout were prepared similarly to the capelin for experiment 1. For the toy treatment, I placed four additional clean pieces of trout (sliced or whole) into four (yellow) 12cm long Sphericon^{TM} 5 dog toys (Figure 4.1). I cut the plastic between three holes in each toy for easier insertion and removal of fish by humans and otters. I then inserted one food item into each toy, leaving one end of the fish protruding from the toy. I placed the baited toys into clean bowls (two toys per bowl). All containers, toys and bowls were washed, bleached and rinsed well after each use.

Figure 4.1 Toy into Which Fish Was Inserted. One fish was inserted into each toy, leaving one end of the fish protruding from the toy.

After the pool was cleaned of leaves, branches, and food (leftovers from the previous day), I set both food bowls down on the sandy ground 50cm from the pool. The distance between the bowls was 90cm. I left the enclosure and released both otters simultaneously into the enclosure, where they remained for the rest of the day.

The camcorder behind the back wall of the otter exhibit recorded otter activities near the food bowl on land and in the water. To avoid influencing the otters' behavior, I remained out of view of the otters for the duration of the recording, other than to check for the presence of food every 15 minutes and to change videotapes after an hour, if necessary.

4.1.3 Analyses

Videotapes were labeled and analyzed as described in Section 2.2.1. I recorded the name of the otter grabbing the food item and his subsequent actions following a predetermined coding of behaviors (Figure 4.2). I calculated Pearson's Chi Square with Yates correction for continuity and used a simulation (with 2000 replicates) to find the p-values (R Development Core Team 2006) to compensate for empty cells. To ascertain possible differences between the otter's behavior towards whole fish vs. sliced fish, I also compared the treatments for whole and for sliced fish separately.

Figure 4.2 Data Recorded for Experiment 2: Handling of Fish Dipped in Sand vs. Fish-in-Toys vs. Free Fish

Time Lift fish = Time otter lifts fish out of bowl for first time.

Mouth or Paw = Body part used to lift fish out of bowl. (M = mouth, P = paw)

ToyTime = Time fish was removed from toy.

ToyLoc = Location at which fish was removed from toy.

5secLocation = Location of fish 5 seconds after time noted in column "Time Lift Fish" with respect to location of fish in bowl.

Water = Whether fish touches the water in the pool anytime before it is eaten. (Y = yes, N = no)

Time Touch Water = Time fish first comes in contact with water.

Time: Bowl-Water = Seconds elapsed between "Time Lift Fish" to "Time Touch Water"

Fish Touched Land = Whether the fish comes in contact with the ground anytime before it is eaten. (Y = yes, N = no)

Submerged Fish = If the fish is taken to water, whether it becomes completely submerged. (Y = yes, N = no)

Swished Fish = Whether the otter swishes the fish in the water (i.e. moves it back and forth with quick motions). (Y = yes, N = no)

Location Swallowed Fish = Location the fish is eaten.

Other Otter lifted Fish Outside of Bowl = Once the fish had been lifted by one otter, did the second otter lift the fish at any time? (Y = yes, N = no)

4.2 Results

Experiment 2 lasted from June to August 2003 and required 27 days of testing. In this

experiment Romulus ate more food items than Remus, 65 vs. 29 items respectively. At

the end of testing Romulus had consumed 20 fish-in-toys, 21 clean fish and 24 sandy fish. Remus had eaten 15 fish-in-toys, 11 clean fish and 3 sandy fish.

Romulus took every sandy fish that he ate to water and submerged it. He carried to water and submerged 19% of clean fish and 25% of fish-in-toys (Figure 4.3). He submerged significantly more sandy fish than either clean fish (Yates Corrected X^2=27.8756, d.f.=1, p<0.001) or fish-in-toys (Yates Corrected X^2=24.0744, d.f.=1, p<0.001). There was no significant difference between his submerging of clean fish vs. fish-in-toys (Yates Corrected X^2=0.0069, d.f.=1, p = 0.934). Romulus swished most of the food items he washed (Figure 4.4), swishing more sandy fish than either clean fish (Yates Corrected X^2=19.8872, d.f.=1, p<0.001) or fish-in-toys (Yates Corrected X^2=16.5525, d.f.=1, p<0.001), but there was no difference between swishing frequency of clean fish vs. fish-in-toys (Yates Corrected X^2=0.3326, df = 1, p = 0.5641).

Romulus ate 41 whole and 24 sliced fish. He washed 3 of 9 (33%) clean whole fish and 1 of 12 clean sliced fish (8%), and he washed 5 of 16 (31%) whole fish-in-toys, but did not wash the 4 sliced fish-in-toys. For both groups (whole and sliced) he washed significantly more sandy fish than either clean fish or fish-in-toys, while there was no significant difference between clean fish and fish-in-toys (at alpha <.01). The comparisons of treatment conditions within groups of whole fish and sliced fish thus showed that Romulus treated both groups similarly.

Remus ate all clean fish and fish-in-toys without washing. He washed one sandy fish before eating, ate one without washing, and began to eat the third (recorded as not washed), then dropped it in the grass. Later he returned, carried the partially eaten fish to the pool, swished it and ate the rest. Remus also washed and ate five sandy fish (not included in analyses), which Romulus had taken and then abandoned. These additional observations are supportive of Remus' inclination to wash sandy food.

Figure 4.3 Number of Food Items Washed by Romulus

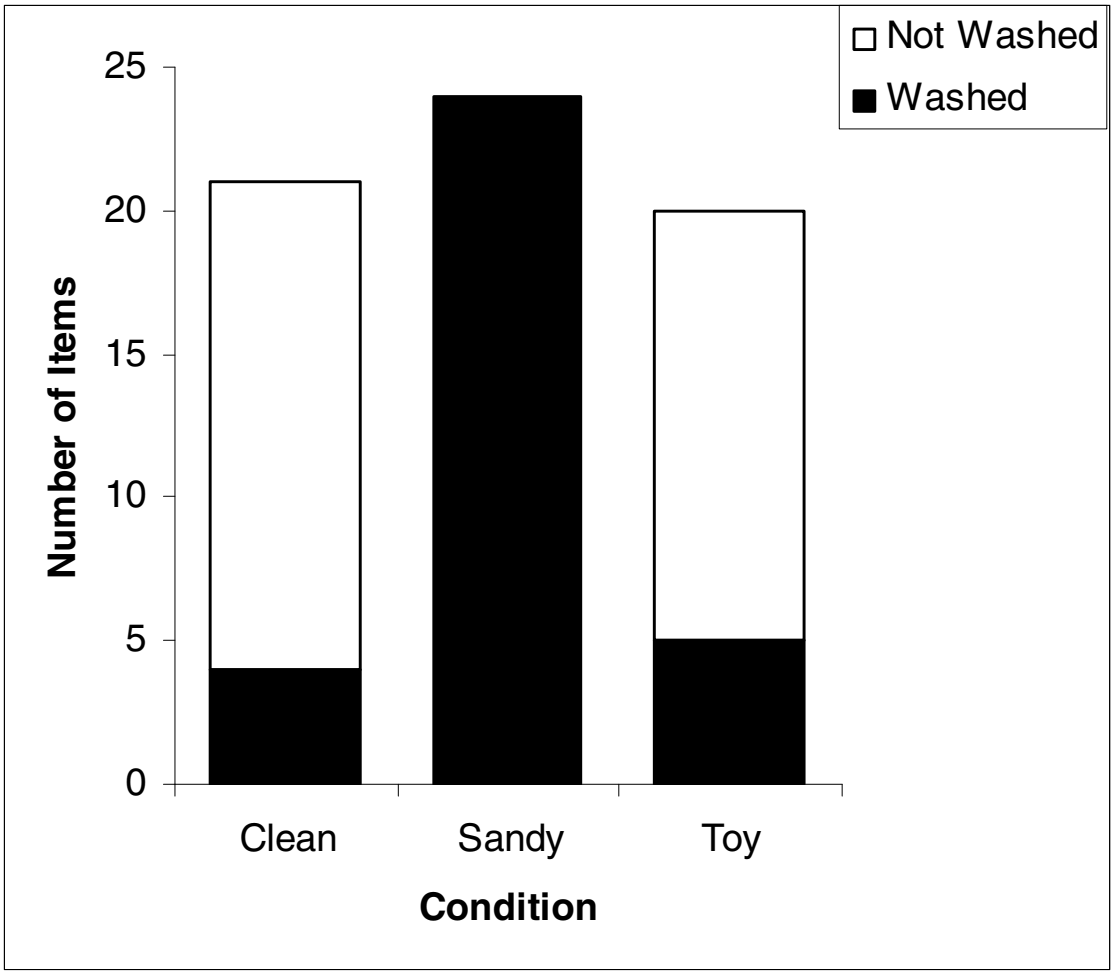
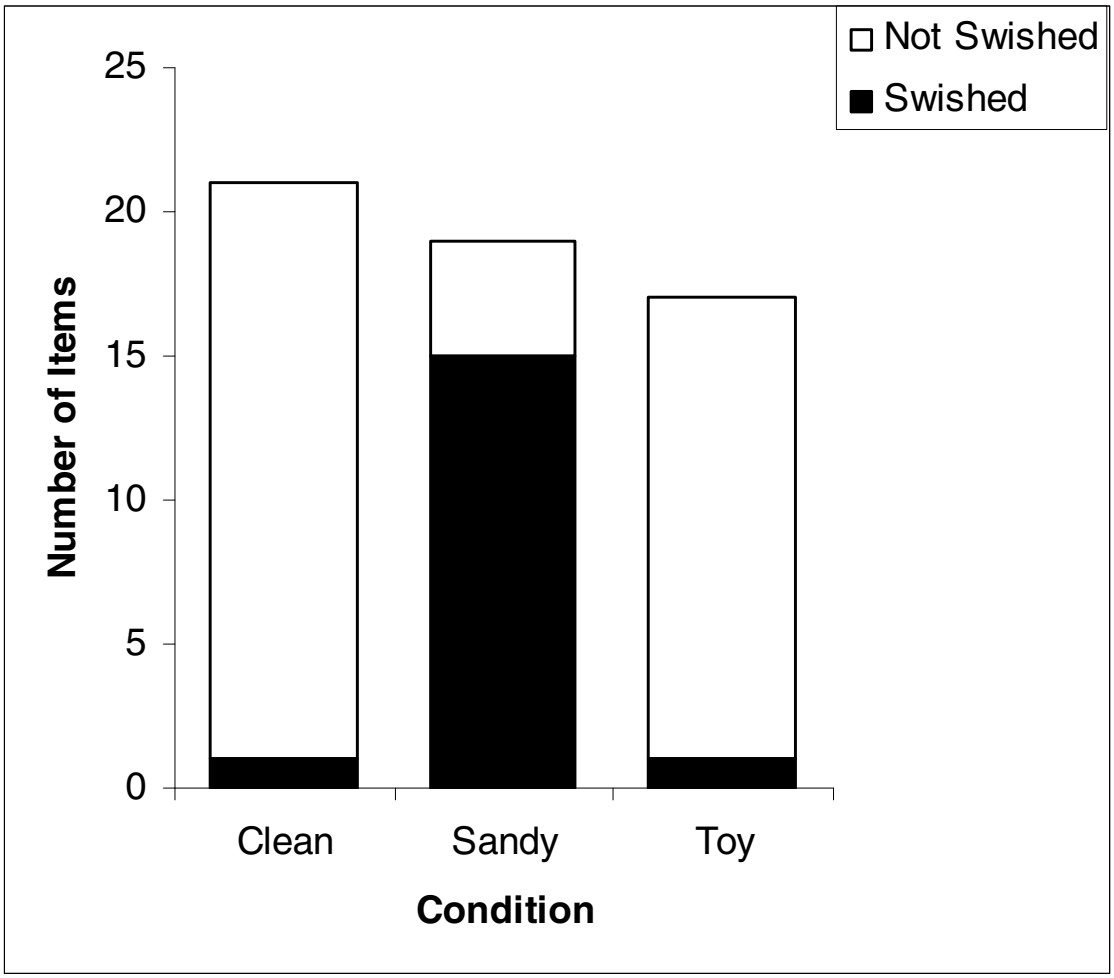


Figure 4.4 Number of Food Items Swished by Romulus



Observations of food manipulations showed that Romulus used his paws extensively during manipulations of sandy food and both otters used their paws for manipulation of food in toys. While Remus usually carried sandy fish to the pool with his mouth, Romulus often used his paws, seeming to avoid getting sand into his mouth. On 7/18/03, a day when sandy fish were offered, I recorded that Romulus "pushed fish around on ground with paw, unsuccessfully tried to push into pool, then left on ground and got next fish; came back after having thrown all fish out of bowls, and pushed fish over pool edge

very carefully, seeming to hold on to it with paw as it slid into the water, ...” Both otters usually removed the fish from the toys by holding a toy with both front paws, while lying on their bellies, and grabbing the fish with their mouths to pull it out. Neither otter ever took a fish to water after removing it from the toy, but Romulus did remove five fish from toys in the water.

4.3 Discussion

Whereas in the first washing test Remus showed a significantly higher frequency of washing, submerging and swishing sandy food vs. clean food, Romulus demonstrated similar behavior in the toy test (experiment 2). Both otters were therefore capable of washing. In the toy test, neither Romulus nor Remus carried significantly more fish-in-toys than clean fish to water, but in this experiment Remus hesitated to wash anything and even ate (or partially ate) two sandy fish without washing. Both otters readily removed fish from toys, while lying on their stomachs on land.

Though statistical tests showed no significant difference between clean fish and fish-in-toys, Romulus did carry some fish-in-toys and some clean fish to water. Observations of his handling of fish-in-toys tempt the observer to conclude that Romulus sometimes carried toys to the pool for easier removal of fish, seemingly after attempts to remove the fish on land proved unsuccessful. Other reasons for carrying both clean fish and fish-in-toy to the pool might have been contamination with sand during lifting from the bowl, social constraints (Visalberghi and Fragaszy 1990), play behavior (Bierens De Haan

1932) or a natural predisposition to douse food in water as was speculated for raccoons (Lyall-Watson 1963). Future testing with food requiring more extensive manipulations may demonstrate whether manipulation requirements may cause an otter to carry food to water also. Such testing would have to be performed with otters much more motivated to eat than Romulus and Remus, since more energy- and time- consuming manipulations may cause mammals to abandon feeding attempts during testing in favor of waiting for less costly meals during regular feeding times (Collier 2005).

In this experiment Remus was clearly reluctant to wash food. In 27 days of testing (9 days with sandy fish), he only took three sandy fish from the bowl and ate them (seemingly avoiding fish that required washing), though he ate 26 fish from the other two treatments. Interestingly, he did wash the five sandy fish abandoned by Romulus. The reason for his change in behavior from the first to the second experiment may have been competition with, or the possibility of losing dunked food to his conspecific, such as has been observed among grackles (Morand-Ferron, Lefebvre et al. 2004) and macaques (Visalberghi and Fragaszy 1990). Visalberghi and Fragaszy also described a capuchin, which “washed readily when her cagemates were removed, but did not wash when they were present” (Visalberghi and Fragaszy 1990). Testing the otters separately in the following experiments limited effects due to competition.

5.0 Experiment 3: Handling Sandy Food With and Without Shells and Clean Food With and Without Shells

With this experiment, I wanted to test the generality of my findings by using other captive otters at other zoos and to further explore whether otters might carry food items to the pool to remove an outside shell. I offered the otters clean food, sandy food, clean food inserted into mussel shells or sandy food inserted into mussel shells on separate days, with the order of treatments randomized. I predicted that, if the otters were predisposed to carry any food object requiring manipulation to water, they should carry sandy food and all food within shells to the pool for removing sand from the food or food from shells. If, however, the otters used water only to wash off loose substances, then they should carry all sandy food (with or without shells) to the pool, but not the clean food (with or without shells).

Initially I planned to test nine North American river otters at four zoos and one aquarium. I reduced this plan and instead tested seven otters at three zoos and an aquarium. At other zoos and the aquarium I substituted capelin for fishballs. In Section 5.1, I describe the experiment with capelin at three zoos and the aquarium, and in Section 5.2 my additional testing with fishballs at Connecticut's Beardsley Zoo.

5.1 Materials and Methods

5.1.1 Subjects and Environment

The otters at the aquarium and two zoos were housed in pairs. Dell was housed alone. Lew, April and Dell, though born wild, presumably had not yet developed the foraging skills necessary for survival.

Though all otter exhibits were viewed by the public, the layouts, sizes and traits of the exhibits varied (Table 2.1 and Figures 3.1, 3.2, 5.1-5.5). The exhibit at Trevor Zoo in New York State seemed most similar to a wild otter's habitat. At this zoo, the otters lived in a fenced-in area containing a natural stream-fed pond and temperate forest, where they could catch wild prey that entered their enclosure. At the other extreme, in their exhibit inside a building at the Maritime Aquarium, the otters walked on artificial rocks and swam in a pool of filtered water and only experienced objects and food items that were intentionally placed into the exhibit by humans. However, even the otters at the aquarium were given toys, real tree branches, and occasionally live food.

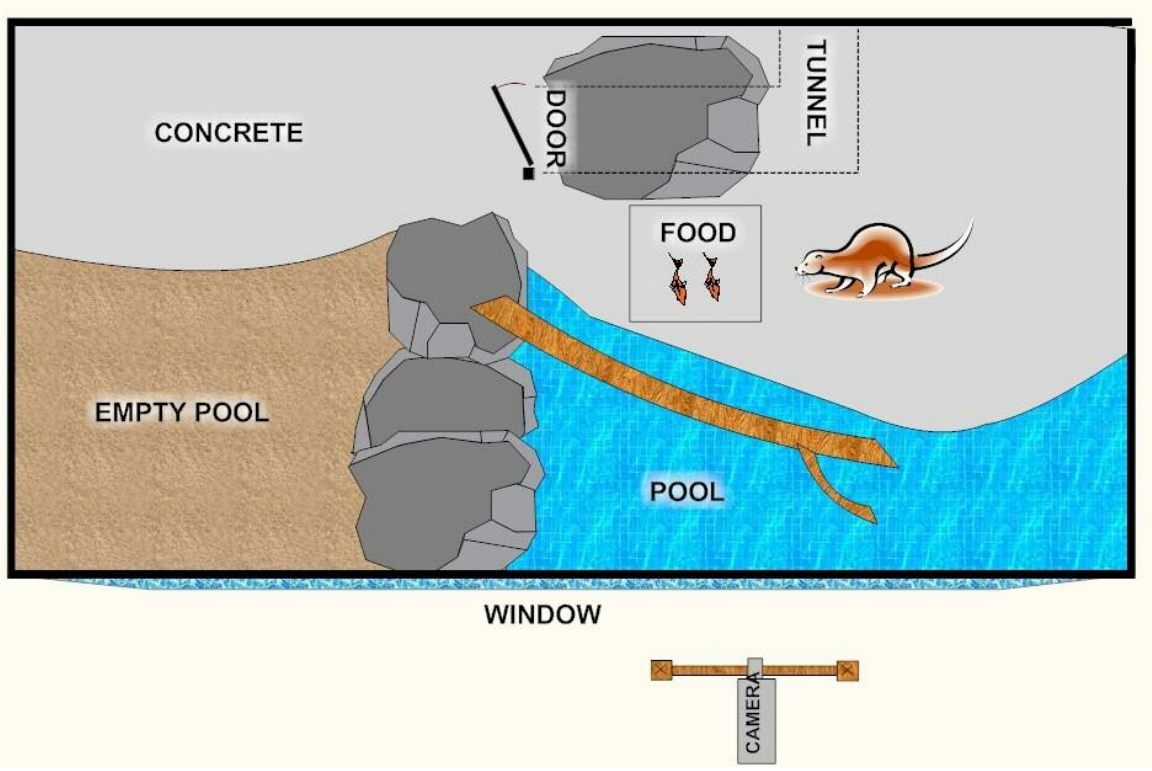
5.1.1.1 Turtle Back Zoo

Romulus and Remus were housed together under the same conditions as during previous experiments (Section 3.1.1).

5.1.1.2 The Maritime Aquarium

During public viewing hours, Lew and Bell were housed together in an enclosed indoor exhibit (Table 2.1, Figure 5.1). While the aquarium was closed, the otters were kept in adjacent cages in a room behind the exhibit. Each cage contained a small bowl of drinking water. The otters entered the exhibit from the cages through a wire mesh tunnel, whose sliding doors were controlled by the trainers. Neither the exhibit nor the overnight cages contained any loose soil or sand.

Figure 5.1 Overhead Schematic of Otter Exhibit at the Maritime Aquarium. The completely indoor exhibit contained two pools of water connected by a small waterfall. Artificial rock formed the bottom and shore along three sides of each pool, while large glass windows bordered the pools on the fourth side. The pools were filled with tap water daily, though the left pool was left empty until after testing. Tree branches were propped into the exhibit. Food was placed (50cm from the pool) on a rocky ledge. I videotaped from the dark hallway. Otter drawing inserted for scale.



The otters normally ate capelin, ground meat ("feline diet"), and occasionally herring filets, fruits or vegetables. They were fed inside the exhibit several times a day and in their cages in the evening. The trainer hand fed them through the top half of a human-sized door about 1m from the pool. Sometimes the trainers offered food within toys or placed live food (such as fish or crabs) into the pools. Food was also placed onto the ground in the exhibit or onto the metal bottom of the overnight cages. Since keepers washed these indoor surfaces daily, food was likely to remain clean until eaten.

Trainers frequently fed the otters in conjunction with behavioral modification. Training consisted of various hand signals, to which the otters were required to respond with specific behaviors (such as lifting a front paw), or of "targeting." Targeting required the otters to touch a target, such as the tip of a stick held in the trainer's hand. The target sometimes required the otter to approach the door or to enter the pool. The otters then received their food reward on land. They had not been rewarded for carrying food to water.

5.1.1.3 Trevor Zoo

The otters Otto and April were housed together. A fenced-in forested outdoor otter enclosure (Table 2.1, Figures 5.2 - 5.4) was divided into a holding area, where the otters were sometimes locked up (at night or during a flood) and a hillside sloping down to the pond. The holding space included a wooden "den" and a tub, which was sometimes filled with water. Above the dam was a lake used as a waterfowl exhibit. Though the otters

were unable to cross the dam to the waterfowl area, small aquatic organisms did fall over the waterfall into the otters' exhibit and the otters often caught fish and other aquatic prey. Heavy rains could conceivably create puddles in the forested area, but the water would seep through between the pebbles on the shore with the test area. One test morning a heavy rain raised the pond's water level and flooded the test area. Flood gates were opened and testing commenced after the water level had receded to normal.

Figure 5.2 Overhead Schematic of Otter Exhibit at Trevor Zoo. The otter exhibit was a fenced-in forested enclosure. It contained the "pool" (a natural stream-fed pond measuring about 60 feet by 100 feet), with water cascading over a dam into the pool at one end and flowing out through grates at the other end. The bottom and edges of the pond consisted of earth and rocks. For testing, the otters ran from the holding area down the forested hillside and swam through the pond to reach the test area. Food for testing was placed (50cm from the water's edge) on large flat slabs of stone. The video camera stood on a bridge above the dam. Otter drawing inserted for scale.

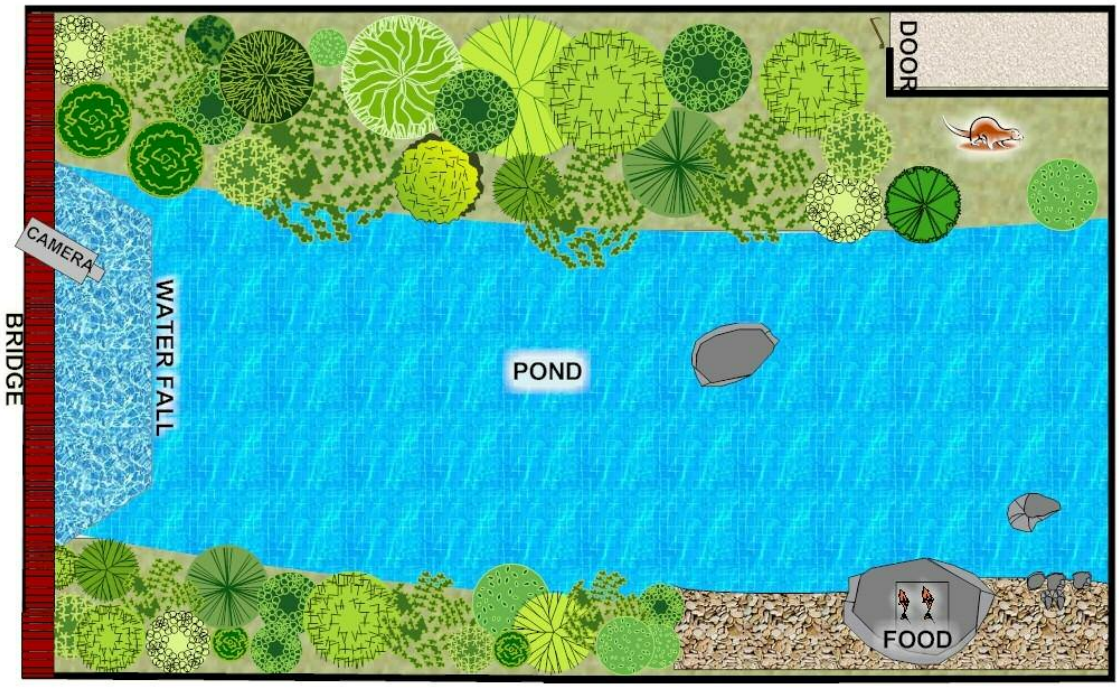


Figure 5.3 Photograph of Upper Section of Otter Exhibit at Trevor Zoo. A dam separated the otter exhibit (in the foreground) from the waterfowl exhibit (in the background). Small aquatic organisms sometimes fell down the waterfall and were preyed upon by the otters. During testing, the video camera stood near the center of the bridge above the dam.

Figure 5.4 Photograph of Pond and Test Area in Otter Exhibit at Trevor Zoo. The shores of the pond included forested hillsides and a beach with pebbles. Food for testing was placed on large stone slabs at the pebble beach (right rear of photo). The otter is circled in red.

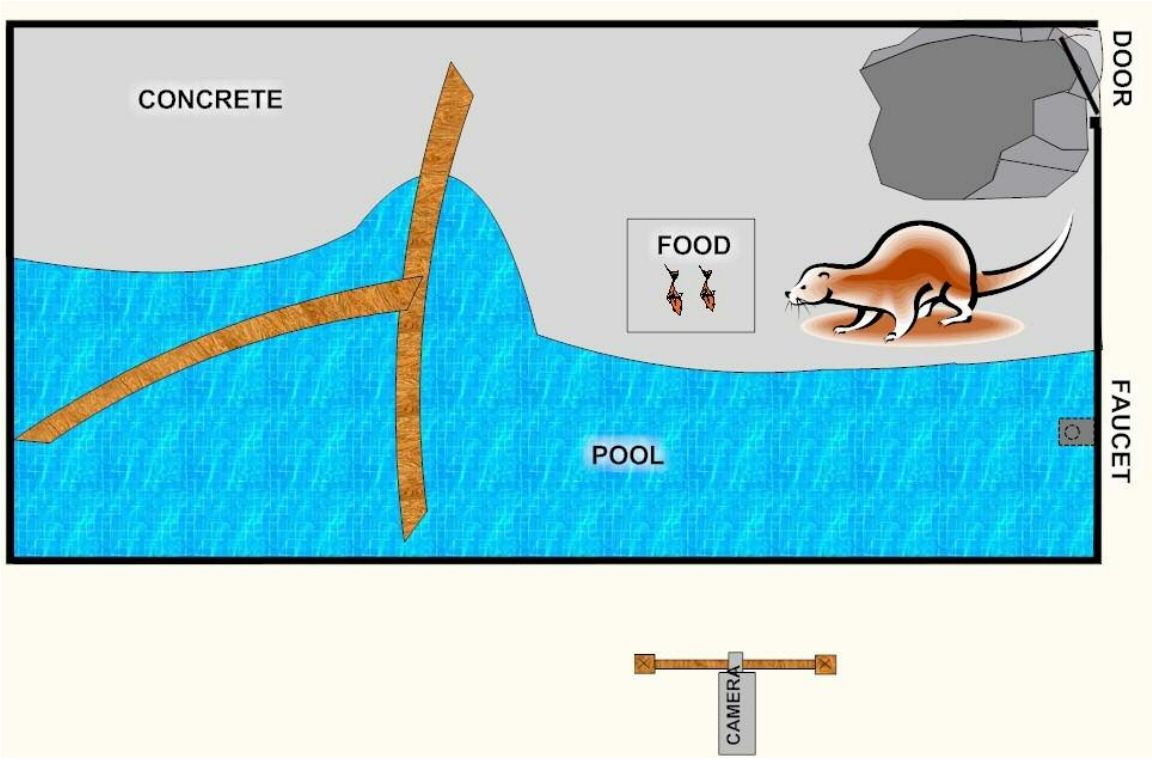
Every morning the otters were fed in the holding area upon ringing of a "dinner bell." Ground meat ("feline diet") or tilapia fillets were placed directly onto the earth in the wooded area, at least 40 feet from the pond and several feet from the water tub.

5.1.1.4 Trailside Museums and Zoo (Trailside Zoo)

Dell was housed alone in an outdoor cage (Table 2.1, Figure 5.5) for approximately seven hours during the day, and was kept in an indoor enclosure from late afternoon until the

next morning. The outdoor exhibit did not contain any loose soil, sand or hay. The indoor enclosure included a trough with flowing water and the floor was covered with hay.

Figure 5.5 Overhead Schematic of Otter Exhibit at the Trailside Museums and Zoo. The cage contained a pool fed from a waterspout. Cement and artificial rock formed the pool bottom and the water's edge along one side of the pool. The other three sides of the pool were of perpendicular cement walls with tight-mesh fencing above and formed the edge of the exhibit. A large tree branch was propped over the pool. For testing, food items were placed (50cm from the pool) on the rock. I hid behind a cardboard blind with the video camera. Otter drawing inserted for scale.



The otter was normally offered food once before 10 AM and once in the late afternoon. He was fed various types of whole fish (depending on availability), dry cat kibble, canned cat food and pieces of fruit. Outdoors, food was usually placed onto the rocky surface within 1m of the pool's edge. This surface was cleaned daily. Indoors food was laid directly on the floor.

5.1.2 Test Item Preparations

For this study, there were four treatments, which were tested side-by-side:

clean food	sandy food
clean food in shell	sandy food in shell

I used two food items per treatment per otter tested on each experimental day. The treatments were randomized.

I bought fresh mussels at a supermarket, boiled them, and scraped the shells clean. To minimize transmission of infectious organisms, I washed the empty shells with dishwashing liquid (Ajax) and rinsed them four times. Then I soaked the shells in bleach solution for 10-15 minutes and rinsed them three times, soaked them in water for 10 minutes and rinsed again. I cut wooden tongue depressors to a width of 1.5cm and placed these into the opening of each shell to prop it open slightly, while leaving the hinge of the shell intact. The shells were dried overnight with a rubber band around each shell to hold the wood in place. The shells were stored in a tin can lined with paper towels. The morning of testing, I removed the rubber band and wood from each shell (four shells per otter). The dry shell remained stiff with a 1.5cm opening (at the widest point).

All capelin (Section 2.2) had been shipped in frozen batches and were defrosted before testing. At Turtle Back Zoo and the Maritime Aquarium I used the capelin provided by the facility and normally fed to their otters. I procured a shipment of frozen capelin to be used at the Trevor Zoo and at the Trailside Zoo. Otters at these two zoos were unfamiliar

with capelin until several days before the start of testing, when they were offered capelin to check for palatability. All otters readily consumed the fish before the start of testing.

On the morning of testing, fish were prepared out of view of the otters. Four capelin per otter were cut in half crosswise at the attachment of the ventral fin. One front half and one back half were used for each treatment. To avoid getting sand on clean capelin, capelin were used in the order described below. Capelin in the "clean food" treatment condition were used without further treatment. Capelin in the "clean food in shell" treatment were placed into shells (one piece of capelin per shell), such that the head or tail faced the opening of the shell and the cut end of the fish was near the hinge of the shell. Capelin in the "sandy food" treatment were dipped in fine aquarium sand (Section 2.2), such that they were covered with as much sand as would stick to the outside of the fish. For the "sandy food in shell" treatment, the food items were first dipped in fine aquarium sand and then inserted into shells.

Otters were tested in their exhibits. At each zoo, a test area was chosen, so that fish could be laid out 50cm from the edge of the pool on a flat surface, in an area easily visible through the camcorder. At the Maritime Aquarium and at the Trailside Zoo this area was within 1 meter of and on the same shore as the doors through which the otters entered the enclosure. On test days at the Maritime Aquarium, waterfalls were turned off and only one of the two pools was filled, such that the otters could only wash in the pool close to the test area. At Turtle Back Zoo, the test area was located at a distance from the entrance doors, such that the otters had a choice to swim through the pool or run along the side of

the pool to the test area. At Trevor Zoo the door from the holding area to the large enclosure was located on the opposite side of the pond from the test area. The otters had to run down the forested hillside and swim through the pond to get to the test area. (Due to the forest on the steeply sloping hillside, the otters' manipulations would not have been clearly visible on the shore close to the holding area.)

The test area was prepared while the otters were locked up out of view. At the Maritime Aquarium and at the Trailside Zoo the food was laid out directly on the cleaned and dried artificial rock. At the Trevor Zoo large flat slabs of stone were placed at the edge of the pond at the pebble beach before the first day of testing. These slabs were rinsed with tap water and blotted dry before each test session. At Turtle Back Zoo, a piece of cleaned and dried Plexiglas was laid on top of the sand each morning before testing. Any puddles of water near the test areas were blotted dry with paper towels. The food items were laid side-by-side on the cleaned surface at a distance of 50 cm from the edge of the pool. Whenever possible, clean items were handled before sandy ones.

5.1.3 Data Collection

On each test day at the Trailside Zoo Dell was tested once. At facilities with 2 otters, otters were separated for individual testing and were tested consecutively (see Table 5.2).

Table 5.2 Example of test schedule (The Maritime Aquarium)

Time Schedule:
(The following time schedule is approximate.)

9:00 AM: Test Set-Up for Lew
(random order -- clean food, sandy food, clean food in shell, sandy food in shell)

9:05 AM: Lew enters exhibit

9:15 AM: Lew re-enters night enclosure
Test Set-Up for Bell
(random order -- clean food, sandy food, clean food in shell, sandy food in shell)

9:20 AM: Bell enters exhibit

9:30 AM: Lew enters exhibit
[End of Testing]

Since the otters at Turtle Back Zoo had never been separated for testing and were unaccustomed to being separated, I prepared them for separation, by training them for several weeks before beginning the experiment. The two otters normally slept huddled together in the middle cage of three adjacent indoor cages. Using a piece of capelin, I coaxed one otter into the adjacent cage to the right and then closed the door between the cages. While observing the otters for signs of distress, I gradually increased the length of time of separation from 1 minute the first day of training to 10 minutes before the first test day.

In spite of this training, these otters and the Trevor Zoo otters sometimes failed to cooperate during testing. They seemed to become savvy with time and would try to reach their food rewards while blocking the doorway, so that I couldn't close it, or they seemed to wait for additional pieces of food before entering. At Turtle Back Zoo, I sometimes

partially opened and closed the door to the outside exhibit to motivate the otter to enter the adjacent cage. At Trevor Zoo, where otters could not be separated within the holding area, I had to entice first one otter to move from the holding area to the test area (while preventing the second otter from running through the door), then reward the otter to return to the holding area after testing. Finally, after setting up the test items for the second otter, I had to allow only the second otter to run to the test area. The otters' personalities were taken into account and I usually tested the more ravenous female otter at Trevor Zoo first, since she readily returned to the holding area. Occasionally, the smaller and quicker female otter squeezed through the otter door instead of the male and she was tested twice in one day. At the Maritime Aquarium, otters were kept in separate cages overnight and experienced trainers released the otters into the exhibit. Since the young male was easier to manipulate, he was always tested before the female.

On test mornings, any scheduled feeding of otters was withheld until after testing. The otters were tested individually, usually one test session per otter on a given day. To train the Trevor Zoo otters to traverse the large distance from their holding area to the test area, their "dinner bell" was rung and a (clean) food reward was placed at the test area several days before testing started. They learned to run to the test area on the first trial.

If two otters were tested, the first otter was released back into the exhibit with the test area 10 minutes after the second otter entered the area for testing (Table 5.2). Both otters remained in the exhibit for the remainder of the day.

This set-up was normally repeated on separate days for a total of 10 times or more per otter (until each otter ate at least 20 food items from each treatment). At Turtle Back Zoo, where feeding *ad libitum* by keepers usually resulted in low motivation to consume test materials, I collected data on 26 separate days for Romulus and 23 days for Remus, in addition to setting up for testing on several days, when no food items were consumed. The test for these two otters was spread out over 4 months. At the Maritime Aquarium Bell was tested for 12 days, while Lew required a 13th day of testing. At Trevor Zoo Otto was tested for 15 days. April escaped to the test area unexpectedly during the first 2 days of testing and was therefore tested twice on each of those days. She was tested for a total of 12 sessions on 10 days. Testing of the singly housed otter Dell at the Trailside Zoo required only 10 days.

5.1.4 Analyses of Videos

I analyzed the videos as for the previous tests, each session requiring viewing at normal speed and in slow motion, often frame-by-frame to record (by hand) exact manipulations and times of manipulations on data sheets (Figure 5.6). This usually took about 3 hours per session (for a total of approximately 350 hours). Various factors influenced the number of times and the speed with which each session had to be viewed, and the time required for analysis. Examples were (1) necessity of keeping track of food items moved, abandoned, and returned to later, (2) simultaneous manipulation of two food items, (3) speed of manipulation and consumption by otter, (4) complexity of manipulations to be recorded.

Figure 5.6 Data Recorded for Experiment 3: Handling Sandy Food With and Without Shells and Clean Food With and Without Shells

Time Move Food = Time otter moves food for the first time.

Food Location = Location of food from observer's viewpoint: on the left (L), second from left (LM), second from right (LR) or on the right (R) side of set-up

Mouth or Paw = Body part used to move food. (M = mouth, P = paw)

Manipulation Time = Time food is removed from shell.

Manipulation Loc = Location at which food is manipulated.

Water = Whether food touches the water in the pool anytime before it is eaten. (Y = yes, N = no)

Time Touch Water = Time food first comes in contact with water.

Time to Water = Seconds elapsed between "Time Move Food" to "Time Touch Water"

Fish Touched Land = Whether the food comes in contact with the ground anytime before it is eaten. (Y = yes, N = no)

Submerged Food = If the food is taken to water, whether it becomes completely submerged. (Y = yes, N = no)

Swished Food = Whether the otter swishes the food in the water (i.e. moves it back and forth with quick motions). (Y = yes, N = no)

Location Swallowed Food = Location the food is eaten.

5.1.4.1 Interobserver Comparisons

The videos from 10% of the test sessions were analyzed by a second observer. To pick the videos to be analyzed, the number of test sessions (in which at least one food item was consumed) for each otter were added. The total for each otter was divided by 10 and rounded to the nearest whole value to give the number of sessions (for each otter) to be

analyzed by a second person. The sessions for each otter were picked randomly and copied onto DVD (see Table 5.3). With the DVD, the second observer received a blank data sheet for each zoo with the columns labeled for data recording, a list of "Coding of Behaviors", verbal instructions on data recording, and a list of the otters, zoos, and dates to be analyzed. The second observer was unaware of the location of treatments, but was told only that some food items had been treated with sand and others had been placed into shells. The quality of the videos did not allow an observer to distinguish between sandy and clean food items. Food items within shells could not always be distinguished from food items without shells. In such cases, however, as the otter removed a food item from a shell, the existence of a shell could be deducted and the left over empty shells could be seen, while the otter ate the food. The data recorded by each observer was compared and an index of concordance was calculated to determine consistency (Martin and Bateson 1993).

Table 5.3 Total Number of Test Sessions and Number of Sessions Analyzed by a Second Observer
Total Number of Sessions represents the number of sessions recorded for each otter, in which at least 1 food item was taken. Number of Sessions Picked was the number of recorded sessions from that otter, which were analyzed by a second person.

Otter	Total Number of Sessions	Number of Sessions Picked
Romulus	26	3
Remus	23	2
Bell	12	1
Lew	13	1
Otto	15	2
April	17	2
Dell	10	1
Total	116	12

5.1.5 Statistical Analyses

Non-parametric statistics were used to analyze the binary response data (washed vs. not washed; swished vs. not swished). When frequencies were small, I calculated Pearson's Chi Square with Yates correction for continuity and used a simulation (with 2000 replicates) to estimate the p-values (R Development Core Team 2006). The assumption of independence in chi-square testing was unlikely to be fully supported by the test conditions, since the data may have been clustered both by trial, facility (zoo or aquarium) and/or individual otter. Factors such as the weather, outdoor noises, and the otter's internal state were likely to be more similar within trials than between trials. I tested for homogeneity of chi-square to determine whether the data from the seven otters could be pooled. Since the two food items per treatment offered at each trial violated independence of data, I performed a separate analysis with only the first fish per treatment taken by each otter in a session.

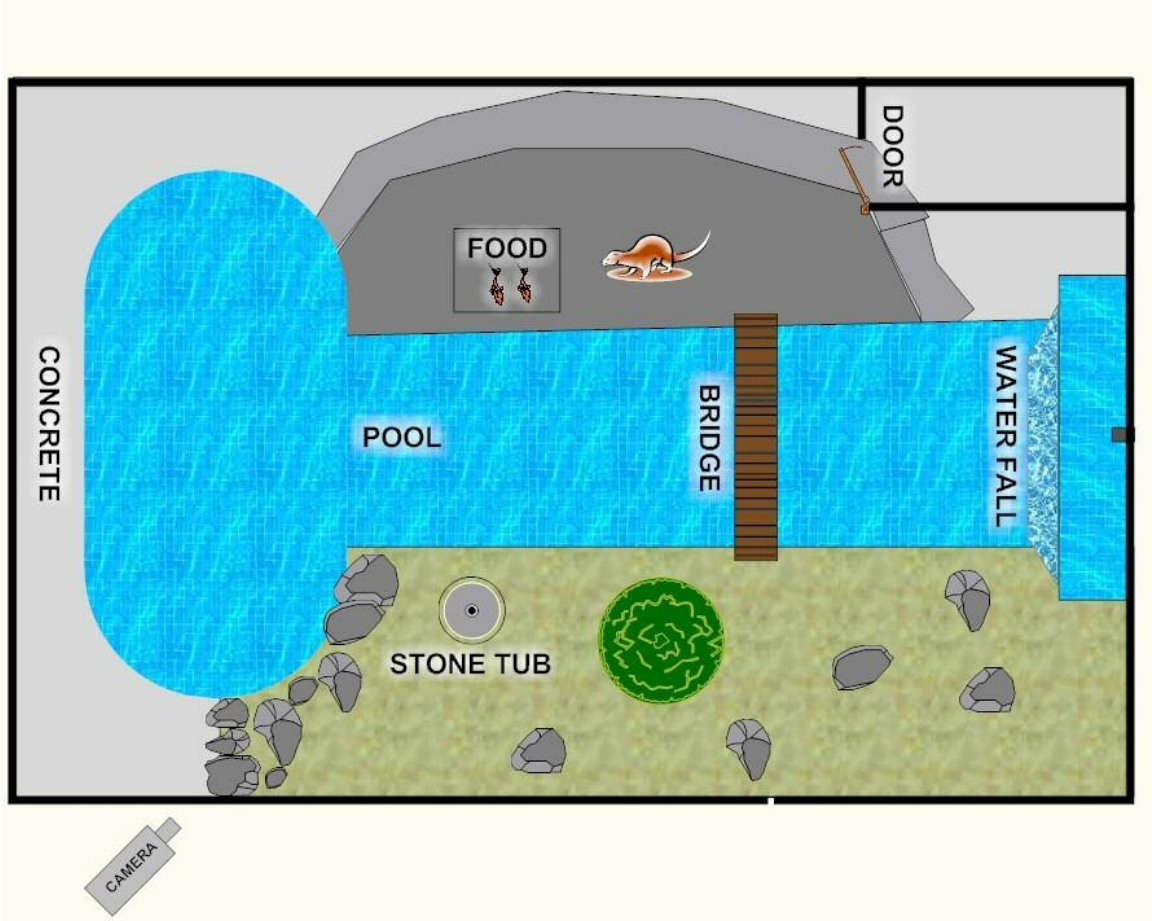
5.2 Handling Clean Fishballs With and Without Mussel Shells and Sandy Fishballs With and Without Mussel Shells

5.2.1 Subjects and Environment

Rizzo and Chewy were housed together in a naturalistic outdoor exhibit (Table 2.1, Figure 5.7) with an adjoining indoor area at Connecticut's Beardsley Zoo. The otters were regularly fed capelin and ground meat ("feline diet"), either thrown into the pool or

hand-fed and received supplements of fruit, vegetables, and sometimes live fish or crayfish.

Figure 5.7 Overhead Schematic of Otter Exhibit at Beardsley Zoo. Water flowed into a small basin at the upper end of the sloping exhibit, then down a waterfall into a pool, and lastly into a ditch at the lower end of the exhibit. One shore of the pool was rocky, the other covered with sod. The otters could move freely between this outdoor exhibit and their straw containing indoor area. For testing, food items were placed (50cm from the straight section of the pool) on the rock. Otter drawing inserted for scale.



5.2.2 Materials and Methods

I used the same general procedure as described in Section 5.1, but tested the two otters simultaneously with four fishballs per treatment (two per otter) set up side-by-side on the

rocky ground on one side of the pool near its bottom end. The treatments were randomized.

clean food	sandy food
clean food in shell	sandy food in shell

Fishballs were prepared as follows: One pound frozen whitefish fillets were defrosted and boiled. After squeezing the water out of the fish, the fish was mixed with two raw eggs and two tablespoons flour. Balls were formed using two round ½ teaspoon measuring scoops and boiled for two minutes. The fishballs were placed into plastic bags and frozen, then defrosted the morning of testing.

Clean fishballs were placed onto the cleaned rock 50cm from the pool without further treatment. Sandy fishballs and fishballs for the sandy-fishballs-in-shells treatment were rolled in a container of fine aquarium sand until coated well. I inserted either clean or sand-covered fishballs into mussel shells to prepare ach of the experimental treatments. To minimize cross-contamination between treatments, all fishballs for the two clean treatments were prepared before fishballs for the two sandy treatments.

5.3 Results

All seven otters tested with capelin washed significantly more sandy fish than clean fish (X^2=250.44, $p<0.001$), carrying 89% of fish from the sandy treatment, but only 4% of clean fish to the pool (Figure 5.8). They also washed more sandy fish from shells (X^2=168.73, $p<0.001$) than clean fish from shells, carrying 91% of the former, but only

20% of the latter to the pool. Yet they also carried more clean fish from shells than clean fish (not from shells) to the pool (X^2=19.76, p<0.001), demonstrating that the presence of a shell did increase the probability of carrying a food item to the pool. Otters took similar numbers of sandy fish vs. sandy fish from shells to the pool (X^2=0.14, p=0.71). All otters submerged every fish carried to water, except Romulus, who did not fully submerge one clean fish from a shell and one sandy fish from a shell.

Figure 5.8 Number of Food Items Washed by Seven Otters at Three Zoos and an Aquarium

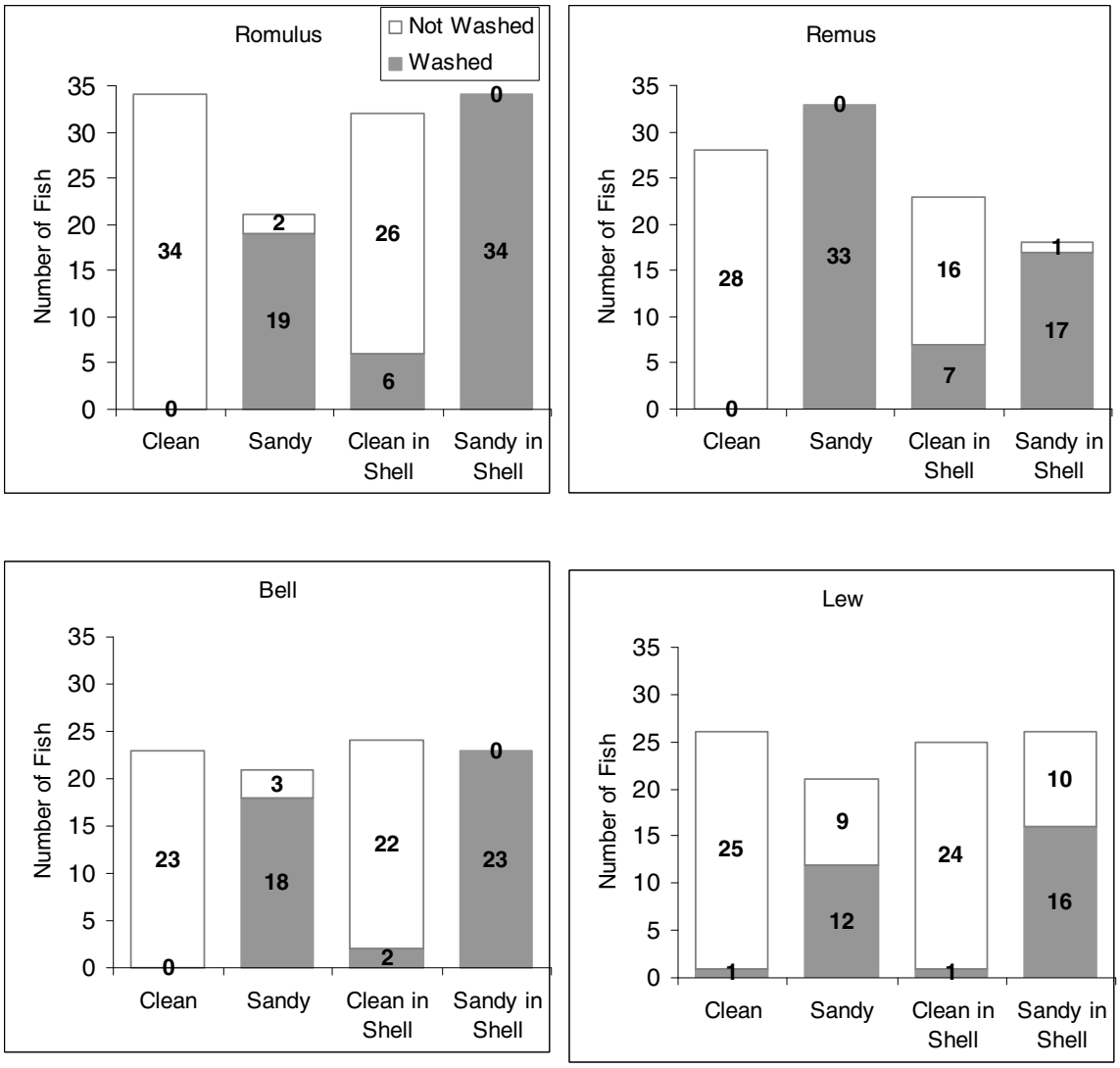
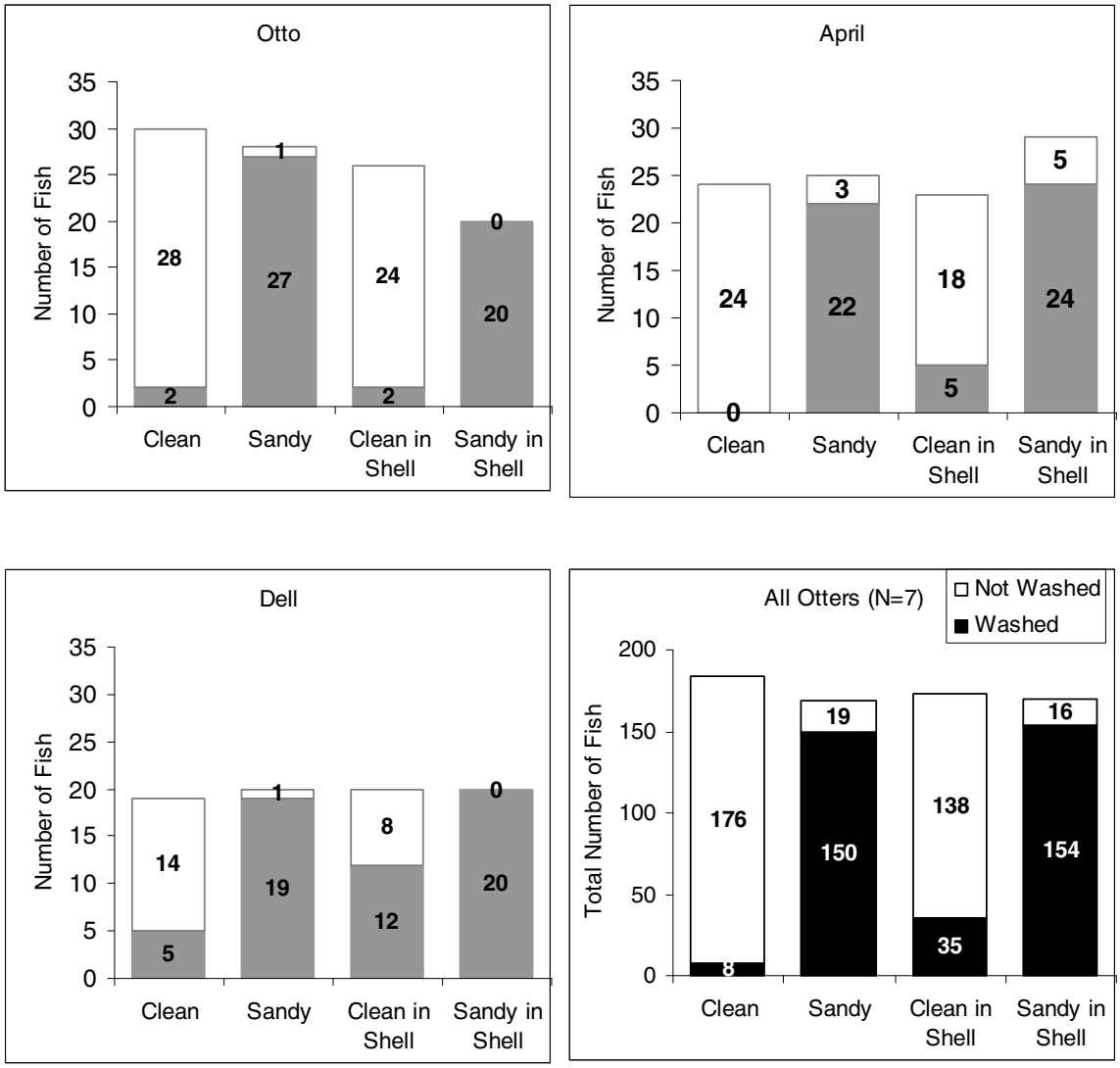


Figure 5.8 (cont.) Number of Food Items Washed by Otters at Three Zoos and an Aquarium



On average, otters swished 74% of all food items carried to the pool. The otters swished significantly more sandy fish than clean fish (X^2=179.83, p<0.001), more sandy fish-in-shells than clean fish-in-shells (X^2=116.79, p<0.001), and more clean fish from shells than clean fish not from shells (X^2=11.61, p<0.001), whereas sandy fish and sandy fish from shells were swished similarly (X^2=0.0036, p=0.95). I isolated those fish carried to the pool (Table 5.4). These differed from the group including all fish in that frequency of swishing between the treatment groups was not significant. Therefore otters had a strong tendency to swish fish taken to water, unrelated to the sandiness of the fish.

Table 5.4 Percent of Fish Swished

Percent Swished	Clean	Sandy	Clean Fish-in-Shell	Sandy Fish-in-Shell	Yates Corrected X^2	P	Simulated P
of fish offered	3.3	73.8	14.0	73.4	301.95	<2.2e-16	5.0e-4
of fish carried to pool	75.0	78.7	68.6	75.3	1.69	0.64	0.66

The otters also manipulated their food in other ways. They rubbed the food against the pool's edge, pushed it through the water, dropped it in the water and retrieved it, held the food with the paws and wiped it with the mouth, and held the food while swimming in circles or rolling in the water. Except for Remus, who entered the water with only 61% of food items washed, the otters entered the water nearly every time they washed food (Table 5.5).

Table 5.5 Percent of Food Items with Which the Otters Entered the Pool

Otter	Percent of food items with which the otter entered the pool
Romulus	96.6
Remus	61.4
Bell	100.0
Lew	100.0
Otto	98.1
April	98.1
Dell	98.2
Rizzo	100

For each treatment, I determined the homogeneity of samples and found that the samples were homogeneous for all comparisons, except for the comparison of clean fish-in-shells to sandy fish-in-shells. Removing Lew from the test resulted in homogeneity for all comparisons. Individual analyses, however, determined that each otter, including Lew, carried more sandy fish-in-shells to water than clean fish-in-shells ($p<.01$ for each individual comparison). Separate analysis including only the first fish per treatment taken in a session confirmed the analysis with all fish included.

I compared the interpretations of two observers. There was a high level of agreement among observers for food location (index of concordance = 0.97), data necessary for the identification of the food items. The index of concordance was also high for water (0.99), submerged (0.99), and manipulation location (0.94), indicating high interobserver agreement. Since observers disagreed about swishing, we repeated the video comparison after a clarification of the definition of swishing. In addition to the original coding (Figure 5.6), we re-defined swishing as follows: "Swishing is any visible sideways motion of the head (at least one head movement to side and back again) with food in

mouth. It may be performed at the water surface (generally associated with splashing) or under water. The otter may be standing on land at the water's edge or it may be standing or swimming in water." New sample videos were watched together and discussed. After this reanalysis, the index of concordance for swished fish increased from 0.79 to 0.90.

I set up test items at Beardsley Zoo for seven days. In four days of testing, Rizzo washed all sandy fishballs and all sandy fishballs-in-shells (Table 5.6), taking more sandy fishballs than clean fishballs (X^2=13.57, p<0.001) to the pool. He did not distinguish between sandy fishballs from shells and clean fishballs from shells (X^2=0.33, p=0.57). He carried more clean fishballs from shells than clean fishballs to the pool (X^2=4.04, p=0.044). He removed only one clean fishball and one sandy fishball from its shell in the pool. Contrary to my expectations, Rizzo frequently removed fishballs from shells by batting the shells around on the rock until the fishballs rolled out. Rizzo submerged every fishball taken to water. He swished none of the clean fishballs, 18% of sandy fishballs, 28% of clean fishballs from shells and 40% of sandy fishballs from shells. Rizzo sometimes rubbed fishballs against the rock on land and in the water, as if trying to rub sand off, thereby causing fishballs to break.

On one of the four days analyzed, Chewy ate a sandy fishball without first washing it. I repeatedly saw him scrounging pieces of food near Rizzo in the water. Once he grabbed Rizzo by the nape of the neck and held onto him while Rizzo ate in the pool.

Table 5.6 Fishballs Washed and Swished by Rizzo

	Clean	Sandy	Clean in Shell	Sandy in Shell
Washed	4	14	7	7
Not washed	11	0	2	0
Total	15	14	9	7
Swished	0	2	2	2
Not Swished	11	9	5	3
Total	11	11	7	5

5.4 Discussion

The washing behavior of seven captive otters at three zoos and an aquarium agreed with the behavior of Romulus and Remus in the first two experiments. This held true despite the different exhibit layouts and histories of the otters. All otters washed sandy fish more than clean fish, indicating an ability and motivation to remove sand from food by washing. Rizzo, with data from only 29 fishballs, also washed more sandy food than clean food. These results demonstrate that captive otters wash sand off food.

Shells increased the likelihood that food would be carried to the pool. However, the presence of sand made it even more likely that food would be carried to the pool than the mere presence of a shell. Captive otters therefore do sometimes carry food to the pool to wash off shells.

Swishing seemed to be inherently linked to washing. Of all items taken to water, sandy food was not more likely to be swished than “clean” food. If a behavior sequence (of

carrying food to water and then swishing) exists for otters, it is more flexible than a fixed action pattern, since only 74% of items carried to water were swished. I observed additional behaviors, which may have helped remove sand such as rubbing the food against hard surfaces, wiping it with the mouth, or moving it through the water in various ways. Though otters may have a strong propensity to swish, washing behavior also seems flexible.

6.0 Experiments 4 and 5: Using an Alternate Source of Water to Wash Food

The goal was to test whether otters would carry food to a source of water other than the pool to wash it. I expected them to wash in the source of water that was closest to the sandy food.

I performed two experiments to determine whether the otters Romulus and Remus, tested individually, would wash sandy food in a tub of water near their pool. In the first experiment, I placed a small shallow tub of water between the food and the pool. In the second experiment I placed a large deep tub of water between the food and the pool, and then varied the location of the sandy food relative to the tub and the pool. I predicted that 1) they should wash food in the tub instead of the pool whenever the food was located closer to the tub than to the pool; 2) they should use the pool and tub equally often when the food was equidistant to the tub and the pool and 3) they should use the pool when the food was closer to the pool than the tub.

6.1 Materials and Methods for Experiments 4 and 5

6.1.1 Subjects and Environment for Experiments 4 and 5

I used Romulus and Remus for this experiment. They were housed under the same conditions as during previous experiments. Their regular feedings consisted of chicken,

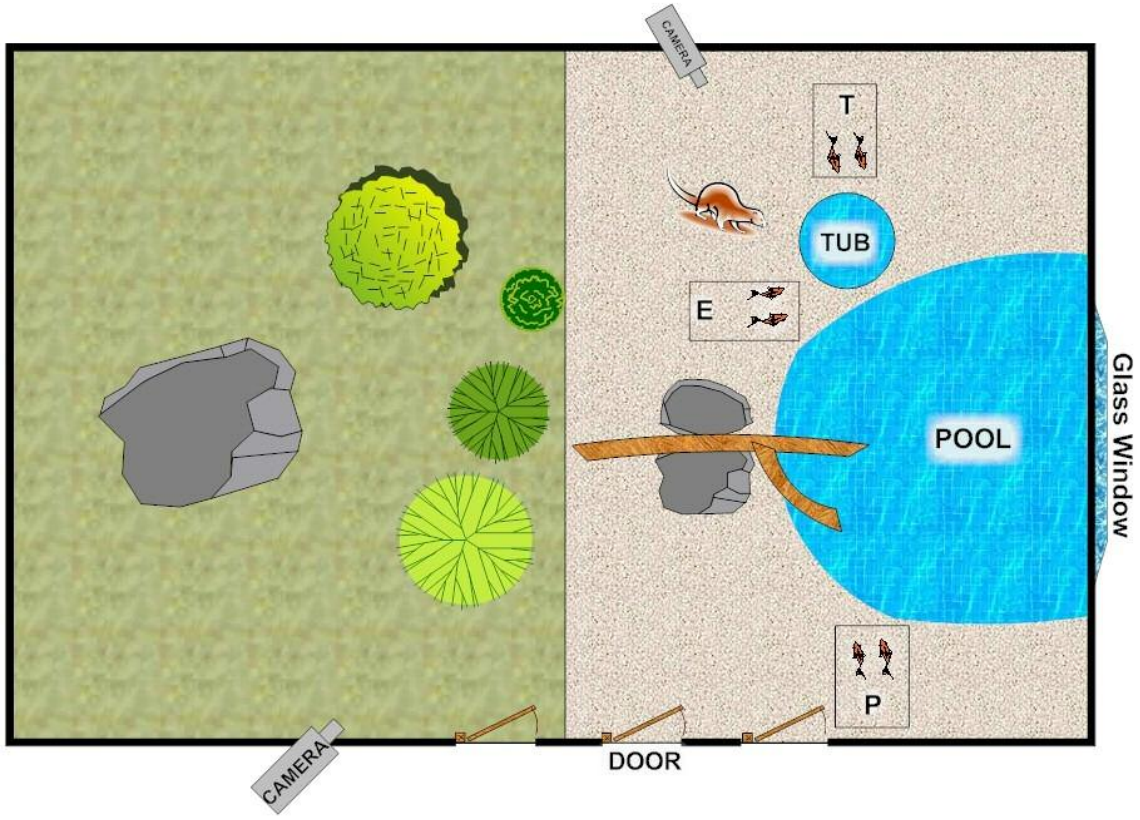
meatballs and capelin, as in experiment 1. The otters were 7 years old during experiment 4, and 8 years old during experiment 5.

6.1.2 Data Collection for Experiments 4 and 5

For the first tub test (experiment 4), I dug a hole 50cm from the pool into which I lowered a round shallow plastic tub (depth: 7cm, inner diameter 33.5cm). The pool's edge consisted of a rounded mound, which I attempted to replicate at the edge of the tub. The tub was close enough to the pool that the otters could stand on top of the mound between the pool and the tub and insert their face into the tub while assuming the same position to insert their face into the pool to wash food. The tub was filled with water from the pool.

Fishballs (Section 5.2.2) were rolled around in a dish with sand and placed into a plastic food bowl. To increase the otters' motivation to eat the food, I offered sandy capelin instead of sandy fishballs on three days. Two pieces of sandy food (fishballs on eleven days, capelin on three other days) were offered in a bowl placed 50cm from the tub at the opposite side from the pool (Figure 6.1). All containers and bowls were washed, bleached and rinsed well after each use.

Figure 6.1 Overhead Schematic of Otter Exhibit at Turtle Back Zoo with Tub as for Experiments 4 and 5. The tub was located 50cm from the pool. For experiment 4, T = Location of the food (50cm from the tub). For experiment 5: T = Location of the food "closer to tub" (50cm from the tub). P = Location of the food "closer to pool" (50cm from the pool). E = Location of the food "equidistant to pool and tub" (50cm from the pool and the tub). On a given day each otter was tested with the food at one location.



I left the enclosure and stayed out of view of the otter being tested. In an attempt to test the otters separately, I first separated the otters into adjacent indoor cages and then let one otter out to the test area. I allotted 10 minutes for the otter to be tested individually and then let the other otter out. Though I had randomized the order of otters to be tested, the focal otter often did not consume the food within 10 minutes and the non-focal otter washed half of the 22 total pieces washed.

For the second tub test (experiment 5), a larger deeper round rubber tub (depth: 23cm, inner diameter: 50.5cm) was lowered into the ground 50cm from the pool. Earth and sand from the hole was piled up around the edges of the tub to match the mound surrounding the edge of the otter pool. I filled the tub (with water from the pool) to a water level (usually a depth of 19cm) at which the distance from the water surface to the top rim of the tub's mound equaled the distance from the pool's water surface to the top rim of the pool's mound (usually 13cm).

Sandy capelin was prepared for the second tub test as for the first washing test. Instead of the food bowl used in the first tub test, I placed a 5mm thick piece of Plexiglas (46cm x 61cm) on the ground. I varied the relative distances between the food, tub and pool to result in three conditions: (1) food closer to the tub (50cm from the tub), (2) food closer to the pool (50cm from pool), (3) food equidistant (50cm) to the tub and pool (Figure 6.1). The order of conditions was randomized and on a given day each otter was tested under one condition. After the pool was cleaned of leaves, branches, and food (leftovers from the previous day), I placed two sandy capelin on the Plexiglas. I left the enclosure and released one otter into the enclosure. The otter remained alone in the enclosure for 10 minutes. To avoid influencing the otters' behavior, I remained out of view of the otters for the duration of the recording.

6.1.3 Analyses for Experiments 4 and 5

Two camcorders were used to record activities (on land, in the tub and in the pool) near each of the three food locations (Figure 6.1). Videotapes were labeled and analyzed as described in Section 2.2.1. I recorded the name of the otter grabbing the food item and his subsequent actions following a predetermined coding of behaviors (Figure 6.2). The test was continued until each otter had consumed 20 food items from each location. To compare locations and to compare frequency of swishing in the pool versus the tub, I calculated Pearson's Chi Square with Yates correction for continuity and used a simulation (with 2000 replicates) to estimate the p-values (R Development Core Team 2006).

Figure 6.2 Data Recorded for Experiments 4 and 5: Using an Alternate Source of Water to Wash Food

Water = Whether food touches the water anytime before it is eaten. (Y = yes, N = no)

Tub/Pool = Whether the food is washed in the tub or the pool (T=tub, P=pool).

Submerged Food = If the food is taken to water, whether it becomes completely submerged. (Y = yes, N = no)

Swished Food = Whether the otter swishes the food in the water (i.e. moves it back and forth with quick motions). (Y = yes, N = no)

Location Swallowed Food = Location at which the food is eaten.

Otter in Water? = Whether the otter enters the water with the food.

Other Otter Lifted Food from Test Area = Once the food has been lifted by one otter, does the second otter lift the food at any time? (Y = yes, N = no)

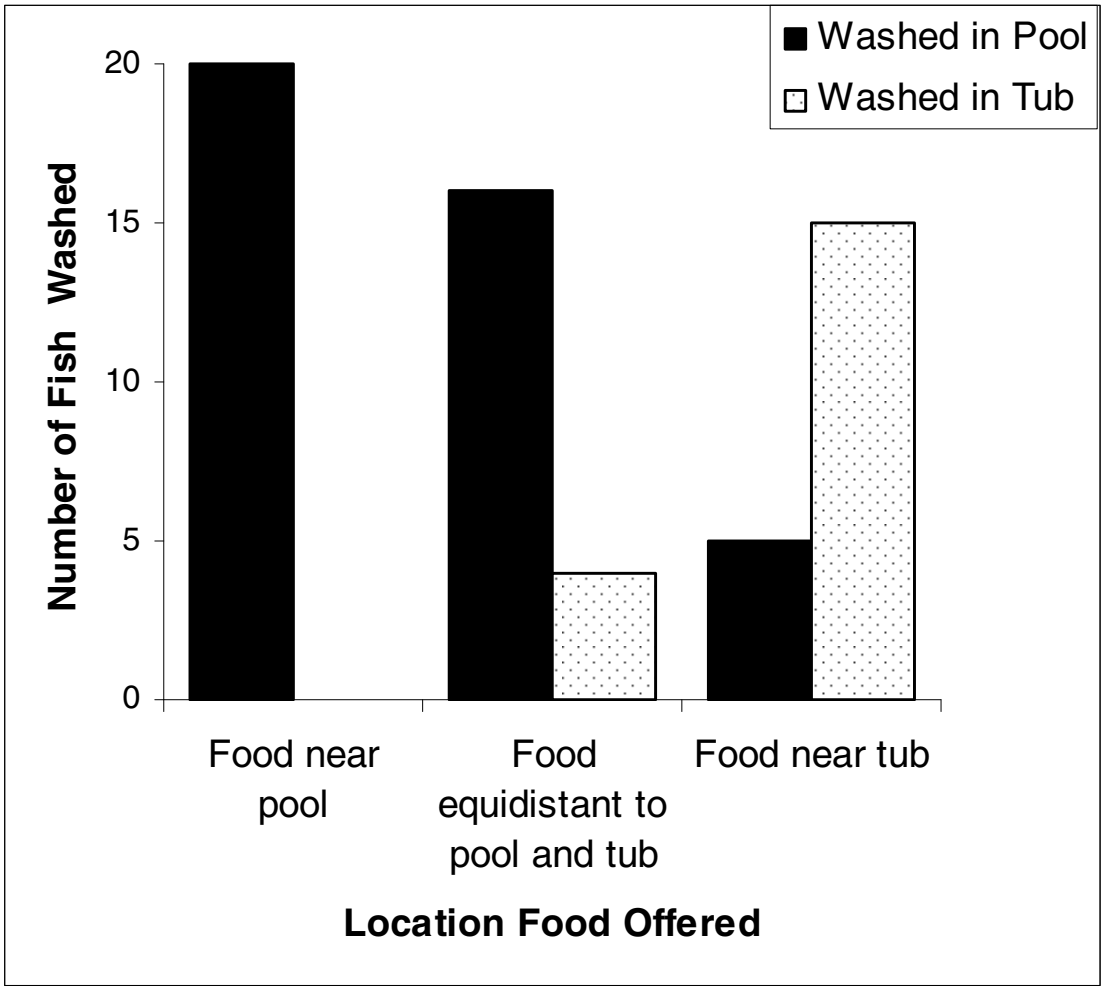
6.2 Results

For experiment 4 (using the small tub) otters were tested for 14 days from July to September 2004. Of 28 food items offered, Romulus washed and ate 14 (9 fishballs, 5 capelin), and Remus washed and ate 8 (7 fishballs, 1 capelin). Remus washed all test items in the pool. Romulus washed and swished one fishball in the tub on the 14th day, while standing on shore at the edge of the tub. However, he took the next fishball to the pool, even though he seemed to invest much effort into nudging this fishball with his nose past the tub into the pool, with the fishball breaking apart as it fell into the water. Romulus and Remus entered the pool with every food item they washed there.

Experiment 5 (using the deep tub) lasted from September to December 2005, with Remus tested on 41 days and Romulus tested on 34 days. Remus washed all fish in the pool, except three, which he did not wash. Romulus washed one of two fish in the tub on the first day of testing (location: food close to tub), taking 2 seconds from grabbing the fish to entering the tub with it. Of all fish offered closer to the tub in experiment 5, he washed 75% in the tub (Figure 6.3). Of fish offered closer to the pool, he washed all in the pool. Of fish offered at equal distance to the pool and tub, he washed 80% in the pool. The number of fish washed in the pool was significantly higher than the number washed in the tub for both food presented near the pool (X^2=20, p=0.000008) and for food presented equidistant to pool and tub (X^2=7.2, p=0.007). The number of fish washed in the tub was higher than the number washed in the pool for fish presented near the tub (X^2=5, p=0.03).

I found no difference (Yates corrected X^2=1.0833, p=0.3) between frequency of swishing for fish washed in the pool (69%) vs. fish washed in the tub (80%).

Figure 6.3 Location of Food Washing (Romulus) in Experiment 5



Romulus entered the water (pool and tub) with 60 of the 64 items he washed. Remus entered the water with 6 of 59 items he washed. Romulus also took 4 fish that were near the tub into the tub and then into the pool, swishing one of the fish in the tub and the other three fish in the pool. This washing data was not used in the analysis, because the data did not show a clear preference for tub or pool, but it leads to interesting

speculations: The tub may have been simply used in transit on his way to the pool, though this is less likely for the one time when he also swished food in the tub before entering the pool. The otter may not have sufficiently cleaned the food in the tub and thus also entered the pool for a more thorough cleaning.

Both otters had clearly noticed the tub early in each experiment, had entered it many times and dipped their faces in it while standing on shore. Any reluctance to wash in the tub was therefore not due to a general lack of using the tub.

6.3 Discussion

Experiments 4 and 5 demonstrated that Romulus, but not Remus, did wash food in an alternate source of water. It took 14 test days for Romulus to "discover" this ability in experiment 4 (small tub) and he might have learned this by trial and error. As I predicted for experiment 5 (large tub), Romulus washed most of the food in the tub when food was closer to the tub. However, he preferred to wash in the pool, when food was at both of the other locations. The similar frequency of swishing fish in the tub and the pool suggests that Romulus did use both locations to remove sand from food.

Features other than the location of the tub might have been too different from the pool for the otters to readily use the small tub to wash food. The otters could not swim in the 7cm deep tub, as they did in the pool. This tub may have been too small for otters to perform many behaviors that aided in washing, such as swishing or dropping and retrieving food.

In the big tub test, Romulus usually entered the tub to wash. Since Remus often did not enter the water to wash, swimming restrictions do not explain his reluctance to use the tub in both experiments. Alternative explanations for reluctance to use the tub might have been that the tub, especially the shallow tub, might have been too small for unobstructed swishing motions or that dirt accumulated more quickly in the tub, hindering a thorough food cleansing. Romulus' laboriously rolling a fishball past the shallow tub to the pool, immediately after washing the previous fishball in the tub, also suggests a strong preference for washing in the pool.

Eleven months after the first tub experiment ended, Romulus carried a fish to a larger tub in the same location without hesitation. I suspect that he not only demonstrated long term memory, but that an internal representation enabled him to associate another type of food with washing in this location. After his first use was inherently rewarded with clean food, his continued use could have been due to the rewarded experience, as in any trial in which behavior connected to an internal representation is linked to a specific stimulus (Shettleworth 2001). More testing with several types of observations would be required to determine the existence of an internal representation.

7.0 General Discussion

To my knowledge, this is the first demonstration of the existence of food washing in a mammalian carnivore. I investigated whether captive North American river otters washed, submerged and swished food items before eating them (experiments 1-3). I also determined whether otters carried food items to water specifically to remove sand. Alternatively, they might use the behavior more broadly to manipulate food items in the water (experiments 2 and 3). My third goal was to determine whether the otters associated washing behavior with a specific location (their pool), or whether their behavior was flexible enough to allow for washing at another location (experiments 4 and 5).

Eight out of nine captive otters washed sandy food by carrying it to water. These results contrasted with those of experiments with raccoons (Lyall-Watson 1963) and grackles (Morand-Ferron, Lefebvre et al. 2004), which dunked food for reasons other than washing. One of two otters tested sometimes washed fish coated with other substances. The otters were housed and tested in different environments, though all received sandy food near a pool of water for testing. All except one entered the water themselves with over 96% of food items washed.

Seven captive otters washed more clean fish when they were from shells than clean fish. This indicated that shells had an effect on the likelihood to wash. Since only 20% of clean fish from shells were taken to the water, otters did, however, effectively remove

shells from food on land. In addition, the presence of sand made it even more likely that food would be washed, than the mere presence of a shell. Captive otters therefore do sometimes carry food to the pool to remove shells, but they prefer to remove shells on land.

In the small tub test, both otters were reluctant to use a small tub as an alternate source of water. I cannot determine if one otter's suddenly washing in the tub on the 14th day of testing was deliberate. A mental transfer of washing to a new location may have occurred, but trial and error learning cannot be ruled out. His immediately washing a fish in the same location nearly a year later suggests long-term memory. The otter's preference for the pool when food items were offered closer to the pool or equidistant to the tub and the pool may have been due to a stronger association to the pool based on past experience, or to features of the pool which made washing in the pool more efficient and/or rewarding.

I observed several behaviors that could have aided in the removal of sand from food. Wild river otters mouthed food, rolled around with it in the water, and also released and retrieved it during foraging (Kruuk 1995). These behaviors may have become adapted to function in food washing, if washing is also performed in the wild. I also observed scrounging of washed food by the older otter at Beardsley Zoo, similar to descriptions of stealing dunked food by monkeys (Visalberghi and Fragaszy 1990) and birds (Morand-Ferron, Veillette et al. 2006), which Morand-Ferron et al. speculated to reduce the prevalence of washing behavior in wild animals. Wild otters might also wash food, but the behavior may be rare due to social constraints.

River otters have evolved to exploit habitats at the border of land and water. They have developed morphological adaptations such as short limbs to accommodate terrestrial and aquatic locomotion (Williams, Ben-David et al. 2002), and unique fur to impede water penetration (Weisel, Nagaswami et al. 2005). Behavioral adaptations that take advantage of these habitats include foraging on aquatic prey near shorelines, traveling on both land and in water (Stokes 1986), and possibly food washing.

If food washing is prevalent among captive otters, why has it not been described more frequently for captive otters and not at all for otters in the wild? I first noticed washing behavior during observations of the one otter in my study who frequently washed food, while conspicuously standing on land at the water's edge. Had he entered the pool, as the other otters usually did, I might not have inferred washing. Some behaviors may be expressed only under certain favorable conditions, as the foraging techniques observed for monkeys and grackles (Visalberghi and Fragaszy 1990; Drea and Wallen 1999; Morand-Ferron, Lefebvre et al. 2004), and also for otters in my experiments 1 and 2, in each of which only one of two otters frequently washed food. River otters are difficult to observe in the wild, so washing could be common, but not yet observed.

Limitations of my study include the lack of a detailed history of the animals and their captive state, which allow for the possibility of human influence on the otters' behaviors. The lack of full control of experimental conditions in a zoo environment increased the difficulty of interpreting results. Yet most animals in zoos are more easily observed than

in the wild and their use in behavioral studies may help us gain insight into potential behaviors, while simultaneously providing enrichment for the captive animal.

Whether or not wild otters wash, cognitively, this study suggests that North American river otters are able to ascertain the need to wash food, can relate objects to substrates in space, and use behavior flexibly to achieve a goal. The long development of otter cubs (Kruuk 1995) and their slow progress in becoming efficient hunters (Polotti et al. 1995, Watt 1993), as well as a possible long-term memory (this study) suggest a long learning period to develop highly complex behaviors. These complex behaviors may be necessary to survive in diverse freshwater and marine habitats and exploit different types of prey according to their availability.

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