

EVALUATION OF DIETARY FEEDING STIMULANTS FOR THE SEA URCHIN
LYTECHINUS VARIEGATUS

A Dissertation

by

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BS, University of Texas in Arlington, 2004

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

MARINE BIOLOGY

Texas A&M University-Corpus Christi
Corpus Christi, Texas

May 2014

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May 2014

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This dissertation meets the standards of scope and quality of
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ABSTRACT

Sea urchins shape their environment primarily through their feeding behavior. For *Lytechinus variegatus* this behavior is largely guided by the detection and response to chemical stimulus. The extent of which compounds and how these specific chemicals influence behavior in sea urchins has received only cursory investigation. The purpose of this study is to characterize the role of several amino acids in the feeding behavior of the sea urchin *Lytechinus variegatus*.

Specimens of *L. variegatus* were collected from Cape San Blas within the Port St. Joseph Peninsula State Park, FL, USA (30 °N, 85.5 °W). Sea urchins were maintained in a semi-recirculating system and fed a standard reference diet (SRD) at ad libitum levels. This diet is used routinely in all sea urchin research at the Shrimp Mariculture Project.

To standardize conditions for testing olfactory and gustatory response, a series of experiments were performed to evaluate the effects of water flow rate and hunger on the chemical response of *L. variegatus*. To characterize the effects of flow rate, feed consumption rate and travel speed was measured while the sea urchins were subjected to different current velocities. *Lytechinus variegatus* was subjected to different lengths of food deprivation to assess the effects of hunger on their feeding behavior. Finally, olfactory and gustatory response was evaluated by exposing *L. variegatus* to one of eight concentrations of six individual amino acids (L-alanine, L-arginine, DL-glutamate, glycine, L-leucine and L-tyrosine).

Results from these studies suggest that *Lytechinus variegatus* is positively rheotactic and under the conditions of the study, increases feed consumption rate with increased flow rate. Also, during periods of prolonged food limitation, *L. variegatus* decreases physical activity but consumes larger amounts of food when it becomes available. *Lytechinus variegatus* also employ food-sourced amino acids to identify food sources and evaluate food palatability.

These findings help expose components of scantily studied mechanisms that drive sea urchin navigation, foraging, distribution, food choice and interactions. These findings could also impact sea urchin aquaculture, affecting both feed formulation and sea urchin husbandry techniques.

DEDICATION

Para ini sa ijo Tay, Nay...

ACKNOWLEDGEMENT

I want to thank my committee members, Dr. Joe Fox, Dr. Addison Lawrence, Dr. Kim Withers and Dr. Lee Smee for the opportunity and guidance in tackling and completing this project. Thank you also to Dr. Stephen Watts and Dr. John Lawrence for the help and support provided me over the years. This dissertation would not have been completed if it were not for the daily help and support of the staff at Texas A&M AgriLife Research—Shrimp Mariculture Project. Jeff Barry thank you, for teaching and helping me make feed and also, for instructing me on the maintenance and the proper care of the sea urchins. I would also like to thank Woody Lawson for caring for the animals when I was not there and even long after I was gone.

This dissertation would not be possible if not for the financial support from the Hispanic Leaders in Agriculture and the Environment (HLAE) fellowship. Dr. Joe Fox, thank you for introducing me into this program and Dr. Frank Pezold, Dr. Greg Stunz and Dr. Deborah Overath for keeping me in it.

I would have been living on ramen if it were not for the constant understanding and support of my laboratory family. Thanks to Ann Marie Quintanilla for the care and the friendship that will always be treasured. Thank you also goes to Milagros and Edgar Tardo for all that they've done for me through the years. Finally, to Rey Ricacho, thank you for the company and the friendship. Those sleepless days fishing in the cold will always be cherished.

Many thanks to my wife, Sheila for the constant support, patience and encouragement even through those times we were apart. Most especially I would like to thank Nanay Gigi and Tatay Bebot. If it were not for them all this would have been but a dream.

Finally, I would like to thank my Father for the inspiration to start on this journey and for the courage and perseverance to see it through.

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INTRODUCTION

Chemical cues are commonly used by aquatic species to regulate vital life processes such as feeding (Hay et al. 1986; Peacor and Werner 2001; Weissburg et al. 2002; Ferner and Weissburg 2005), reproduction (Sato and Goshima 2007), and habitat selection (Zimmer-Faust and Tamburri 1994; Pawlik 1992). Collaborations of scientists with engineers have led to advancements in our understanding of how chemical signals are transported in water (Webster and Weissburg 2009; Hay 1996); however, our knowledge of which compounds and how these specific chemicals influence behavior is still lacking (Hay 2009). For example, our understanding of how chemicals affect feeding behavior has received only cursory investigation (e.g. Hay et al. 1986; Klinger and Lawrence 1985; Dworjanyn 2007). This dissertation focuses on characterizing the role of several amino acids in the feeding behavior of the sea urchin, *Lytechinus variegatus*.

Lytechinus variegatus

Lytechinus variegatus is an echinoid that occurs in a variety of shallow water habitats along the U.S. Atlantic coast from North Carolina to Brazil. It is usually found in beds of submerged vegetation including *Thalassia testudinum*, *Cymodocea filiforme* and *Halimeda* sp. It can also be found on hard substrates that are covered with algae, on mussel beds, shell hash, sand and exposed limestone beds.

Distribution and abundance of *L. variegatus* is influenced by several factors, but most importantly salinity and temperature. Some argue that abiotic factors exert greater control over distribution of populations of *L. variegatus* than biotic factors. In St. Joseph's Bay, Florida, *L. variegatus* survives at 11-35 °C (Beddingfield and McClintock 2000); however, mass mortality events have been reported even when temperatures fall within this range. This indicates that

lethal thermal limits can be greatly influenced by thermal history of individuals and/or other stressors. In terms of salinity, *L. variegatus* does not regulate the osmotic pressure of its coelomic fluid; hence it cannot thrive in lower salinity areas. Mass mortalities have been reported after heavy rain falls and hurricanes (Beddingfield and McClintock 1998). These mass die-offs become more frequent when acute change in salinity is coupled with extreme temperature flux. Mass mortality events significantly reduce natural spawning and recruitment (Beddingfield and McClintock 2000).

Lytechinus variegatus is fast growing. Post-metamorphic juveniles increase diameter from 0.42 to 8.40 mm in 36 weeks and reach 23 mm in just 84 weeks of laboratory culture (Moore et al. 1963). In the field, individuals grow from an initial diameter of 12 mm to 55 mm within 1 year. They achieve sexual maturity at about 40 mm, with an estimated life span of approximately three years (Moore et al. 1963).

Lytechinus variegatus is omnivorous, consuming a variety of plant and animal matter. Turtle grass, *Thalassia testudinum*, is most commonly consumed; however, *L. variegatus* will also consume other seagrasses including *Cymodocea filiforme* and *Halodule wrightii*. Decayed blades of *T. testudinum* are preferentially consumed (Beddingfield and McClintock 1998). Detrital blades with epiphytes are preferred over non-epiphytized blades or even fresh blades. Epibionts on the seagrass are preferred over the seagrasses (Beddingfield and McClintock 1998). At locations in which *L. variegatus* and mussels co-occur, the urchin will consume the whole mussel (Sklenar 1994). Such behavior suggests that selection of diet by *L. variegatus* is a balance of both availability and palatability of food sources.

Lytechinus variegatus was chosen for this research because it was readily available, easy to care for and has been widely studied (Watts et al. 2007; Klinger and Lawrence 1984, 1985;

Lawrence et al. 2003; Beddingfield and McClintock 2000; Gibbs et al. 2007, 2009; Richardson et al. 2011). The macro-nutrient requirements of this species are known (Hammer et al. 2004, 2006a, 2006b; George et al. 2001) and a nutritionally-replete artificial diet has been developed specifically for this species (Gibbs et al. 2007; Hammer et al. 2006a, 2006b). More importantly for the present study, the feeding responses of this species have been characterized (Klinger and Lawrence 1984) and provide a basis for comparison with experimental results.

Chemoattraction

Feeding of aquatic organisms is accomplished through a series of behavioral responses. These responses are mediated by one or a combination of visual, tactile, and chemical stimuli (Lindstedt 1971; Lee and Myers 1996). The term, “chemoattraction,” refers to the cascade of behavioral responses to chemical stimuli that ultimately lead to feeding. Each step in this cascade can be a separate or isolated event, influenced by a particular set of conditions, but can also be considered related to the single act of feeding (Lindstedt 1971).

A model describing the steps in this cascade was described by Lindstedt (1971) and is shown in Fig. I.1. This model, which subdivides the role of chemical modulators into descriptive categories or steps in an overall process, was subsequently elaborated upon by Lee and Myers (1996) to apply to crustaceans. As shown in Fig. I.1, repellents, attractants, and arrestants are compounds or chemicals which exert their influence from a distance (Lee and Myers 1996; Lindstedt 1971), whereas substances affecting taste and gustation can only function upon contact and are referred to as either incitants or suppressant depending on their function (Lee and Myers 1996; Hay 2009). A chemical can be an attractant from a distance but a deterrent upon contact. Past studies focused on either olfaction and chemotaxis (e.g. Ferner and Weissburg 2005; Hay et al. 1986; Klinger and Lawrence 1985) or gustation (e.g. Deal et al., 2003; Dworjanyn et al. 2007;

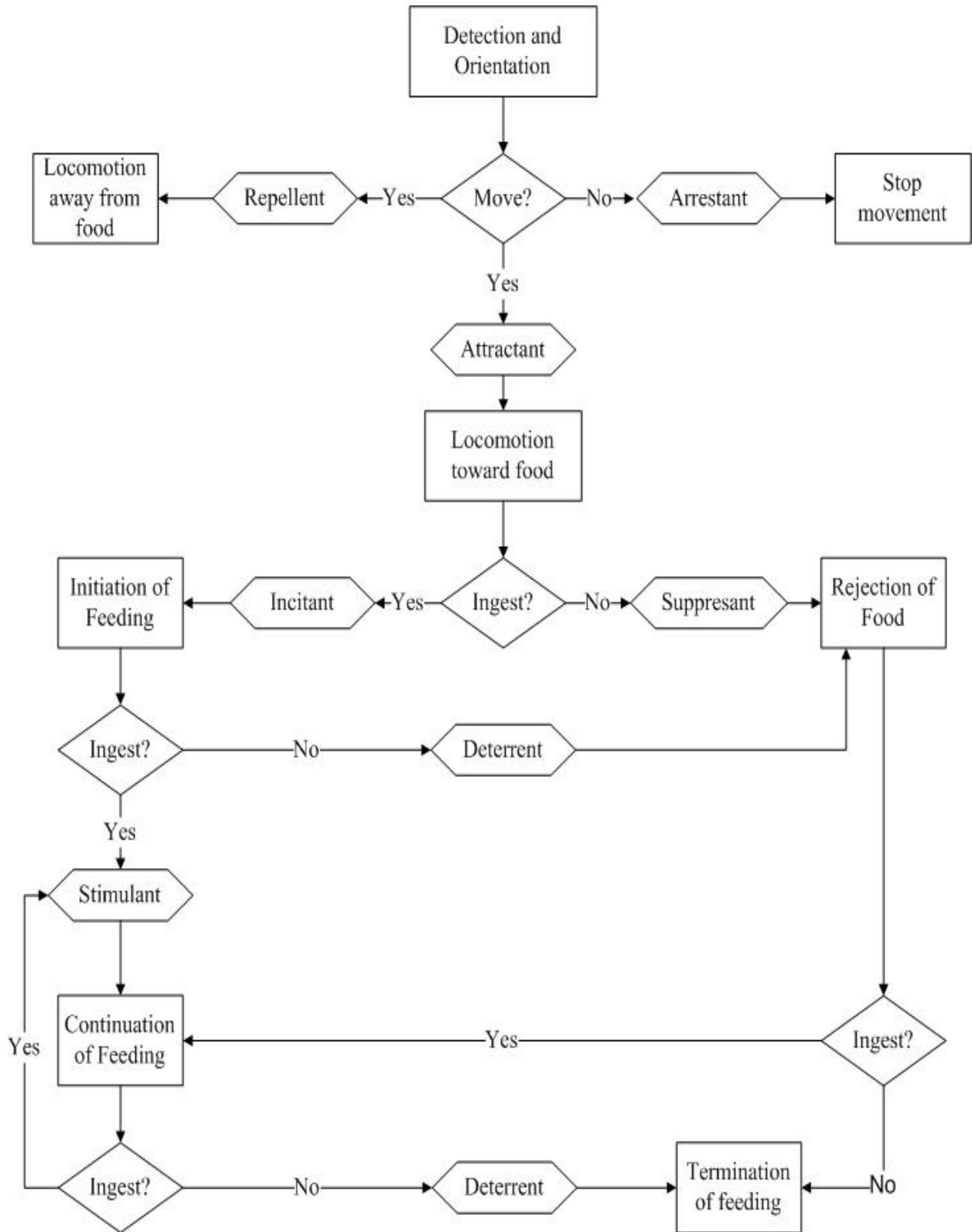


FIGURE I.1. Feeding model for classifying chemical stimuli. Adapted from Lindstedt (1970).

Hay et al. 1986; Klinger and Lawrence 1984), and have provided preliminary information regarding the finer distinctions contained within the Lindstedt model.

Classification of a behavioral response to a chemical stimulus is also contingent upon the direct or indirect guidance of movement towards or away from the source. Kinesis (Webster and Weissburg 2009) refers to an indirect response where the movement is inefficient and unrelated to the direction of the stimulus (e.g. tracking in bacteria [Dusenbery and Snell 1995]), whereas - taxis refers to a directed movement towards or away from a stimulus (e.g. tracking in fishes [Hara 2006]). Response to a stimulus can also be described with prefixes such as: tropo- which describes evaluation of simultaneous sensory inputs over a sensor array and klino- which describe the evaluation of consecutive chemical cues over a series of points in space and time (Webster and Weissburg 2009; Burr 1984).

Aquatic organisms have adopted various strategies to enable successful and efficient navigation for various flow types (e.g. rheotaxis) (Weissburg and Zimmer-Faust 1994; Moore and Grills 1999; Atema 1995; Zimmer-Faust 1987; Wellnitz et al. 2001). Many organisms use flow direction as a starting point in their search since chemical signals are typically entrained in currents. Others use flow to hide their odor from their prey or from a predator (Wisenden 2000; Kats and Dill 1998; Webster and Weissburg 2009; Ferner and Weissburg 2005). It has also been suggested that some organisms use flow to avoid competition (Ferner and Weissburg 2005).

High-velocity and turbulent flows mix and dilute chemical signals, distributing them rapidly over relatively large areas without any distinct borders (Webster and Weissburg 2009; Weissburg 2000). In contrast, low-velocity flows typically create odor plumes with discreet edges and concentrated chemical signals. High-velocity, turbulent flow mixes and dilutes chemical signals, distributing them rapidly over relatively large areas without any distinct

borders (Webster and Weissburg 2009; Weissburg 2000). In contrast, low-velocity flows typically create odor plumes with discreet edges and concentrated chemical signals. Fast-moving, tropotactic organisms with wide sensor spans are typically more successful foraging and navigating in relatively low velocity flows (Webster and Weissburg 2009; Burr 1984). These organisms remain within the odor plume by quickly moving between its edges. In contrast, slow-moving klinotactic aquatic organisms detect chemical signals by integrating successive samples over time, giving them advantage in navigating in fast turbulent flows (Byron and Smee 2012; Ferner and Weissburg 2005). Several studies have investigated chemical navigation in crabs (Jackson et al. 2007; Ferner and Weissburg 2005; Weissburg and Zimmer-Faust 1994), lobsters (Atema and Gomez 1996; Koehl et al. 2001), fish (Carr et al. 1996; Adams et al. 1988), and crayfish (Moore and Grills 1999), all relatively fast-moving organisms with wide sensor arrays. Studies of chemical navigation in slow-moving animals are few (Byron and Smee 2012; Ferner and Weissburg 2005; Ferner et al. 2009), and fewer still addresses the navigation strategies of echinoids (e.g. Pisut 2004).

Chemoattraction can be studied using behavioral (Byron and Smee 2012; Hay et al. 1986; Vadas 1977) or electrophysiological techniques (Binyon 1972; Verheggen 2007) or a combination of both (Kidawa 2005; Barlow 1990). Electrophysiology involves the measurement of voltage change or electric currents in biological specimens ranging in size from ion-channel proteins (e.g. Fertig et al. 2001; Restrepo and Boyle 1991) to whole organs (e.g. Croll 1983). Electrophysiology has been used to study chemoreception in a wide range of animals including catfish (Suzuki and Tucker 1971; Restrepo and Boyle 1991), asteroids (Kidawa 2005, 2006; Binyon and Hasler 1970; Binyon 1972), ophiuroids (Moore and Cob 1985), lobsters (Grundfest et al. 1959; Ache et al. 1976), and several other organisms (e.g. Verheggen 2007, Lawn 1975,

Haseeb and Eveland 1991). Responses observed in these studies were generally characterized using non-linear regression techniques. In contrast to ANOVA techniques, this allows for superior characterization of the responses and the estimation of the concentration that elicits the measured response. Several compounds and their associated receptors have been identified via electrophysiological techniques (Su et al. 2009; Chandrashekar et al. 2006). Ecological significance and impact in electrophysiological studies are not easily discernible however, since the animal's interpretation of the stimuli to behavior cannot be readily observed (Zimmer-Faust 1989; Lindstedt 1971).

Behavioral assays evaluate changes in behavior in response to chemical stimuli (Byron and Smee 2012; Koehl 2001; Hara 2006; Lindstedt 1971). Behavioral assays allow evaluation of the whole suite (e.g. antennule flicking in lobsters [Koehl 2001] and chemotaxis in fishes [Hara 2006]) of responses of an organism to a stimulus. Most behavioral studies use only one to four concentrations of a compound (e.g. Hay et al. 1986; Klinger and Lawrence 1984). Generally, these concentrations are at the extreme ends of ecological significance. Also, the compounds studied are typically complex mixtures (i.e. soaks and extracts [Dworjanyn et al. 2007]) or artificial mixes of several compounds at different ratios (Kidawa 2006). These techniques allow only for qualitative assessment of a response to a compound. As seen in electrophysiological studies, concentration is as important as compound identity in characterizing behavioral responses. Improved understanding of hydrodynamic forces that carry chemical signals has afforded standardization of the physical (i.e. turbulence, flow rate, etc.) components of behavioral assay test systems (Weissburg 2000; Yen 1998; Koehl 2006). Still, behavioral assays that test the effects of specific compounds in graduated concentrations are limited.

This study evaluated the response of *L. variegatus* to several amino acids introduced in graded concentrations. For a more thorough description of chemoattraction in *L. variegatus*, the effect of water flow rate and hunger on chemotaxis and consumption rate in *L. variegatus* were investigated.

Chemoattraction in Sea Urchins

Several studies have shown that sea urchins are capable of chemical perception from a distance (teloreception); however, few have demonstrated the use of individual compounds as chemoattractants (Vadas 1977; Hay et al. 1986). Using a Y-maze test system, Vadas (1977) showed that *Strongylocentrotus drobachiensis* and *Strongylocentrotus franciscanus* could be attracted from over 100 cm away to extracts of the kelp, *Nereocystis luetkeana*. *Arbacia punctulata* has been observed to move towards palatable species of algae, yet avoids or is not attracted to algae possessing chemical defenses (Hay et al. 1986). Another species, *Diadema antillarum*, forages on coral reef algae or in seagrass beds at night, but seeks shelter in the same crevice during the day indicating a homing ability possibly facilitated by chemical cues (Carpenter 1984).

When offered a choice of natural diets (e.g. algae), sea urchins display a hierarchy of preference (Prince and LeBlanc 1992; Beddingfield and McClintock 1998; Hay et al. 1986, Vadas 1977; Vadas and Elner 2003). Chemotaxis and consumption are positively correlated in *A. punctulata* (Hay et al. 1986); however, movement toward potential food items should not be considered a direct measure of the palatability of the food (Hay et al. 1986; Lee and Myers 1996; Lindstedt 1971). Feeding preference has been attributed to several factors including the physical properties of the food as well as presence or absence of attractants or deterrents (Lee and Myers 1996; Nagi and Kaneko 1975; Sakata 1989; Hay 1996; Deal et al. 2003; Hagen et al., 2002;

McConnell et al. 1982). In urchins with generalized food preferences (e.g., *L. variegatus*), ease of ingestion and availability of food are believed to be an important factor determining food selectivity (Lawrence 1975; Hay et al. 1986). Several studies have shown that sea urchins will consume foods with low nutritional value when given no other choice and even consume algae with toxic defensive compounds (Deal 2003; Hagen et al. 2002; McConnell et al. 1982). Food preference appears to be a compromise between availability and palatability and not related to the caloric or nutritional value of the food (McConnell et al. 1982; Hay et al. 1986; Beddingfield and McClintock 1998).

Individual chemical compounds have not been well investigated as feeding stimulants for sea urchins. Glycerolipids in soy lecithin act as feeding stimulants for *Strongylocentrotus intermedius* (Sakata et al. 1989). On the other hand, similar compounds from the same group can be feeding deterrents to *A. punctulata* (Deal et al. 2003). Consumption of agar by *L. variegatus* was increased by the addition of glucose and L-phenylalanine but not by other amino acids, other sugars, or by other compounds including a range of proteins (Klinger and Lawrence 1984). When consumption of an artificial diet by the sea urchin *Strongylocentrotus pulcherrimus* ceased, feeding was reinitiated by the addition of a water soluble extract from the brown algae, *Undaria pinnatifida* (Nagi and Kaneko 1975). Extracts from co-occurring marine plants were observed to be feeding stimulants in a basal, artificial diet for *Tripneustes gratilla* (Dworjanyn et al. 2007).

Sea Urchin Fisheries and Aquaculture

Global sea urchin production has been in decline since it peaked in 1995. In most countries, domestic production had diminished even prior to 1995 (Fig. I.2). This is most evident in Japan which dominated sea urchin production since 1950. Japanese production peaked in 1969

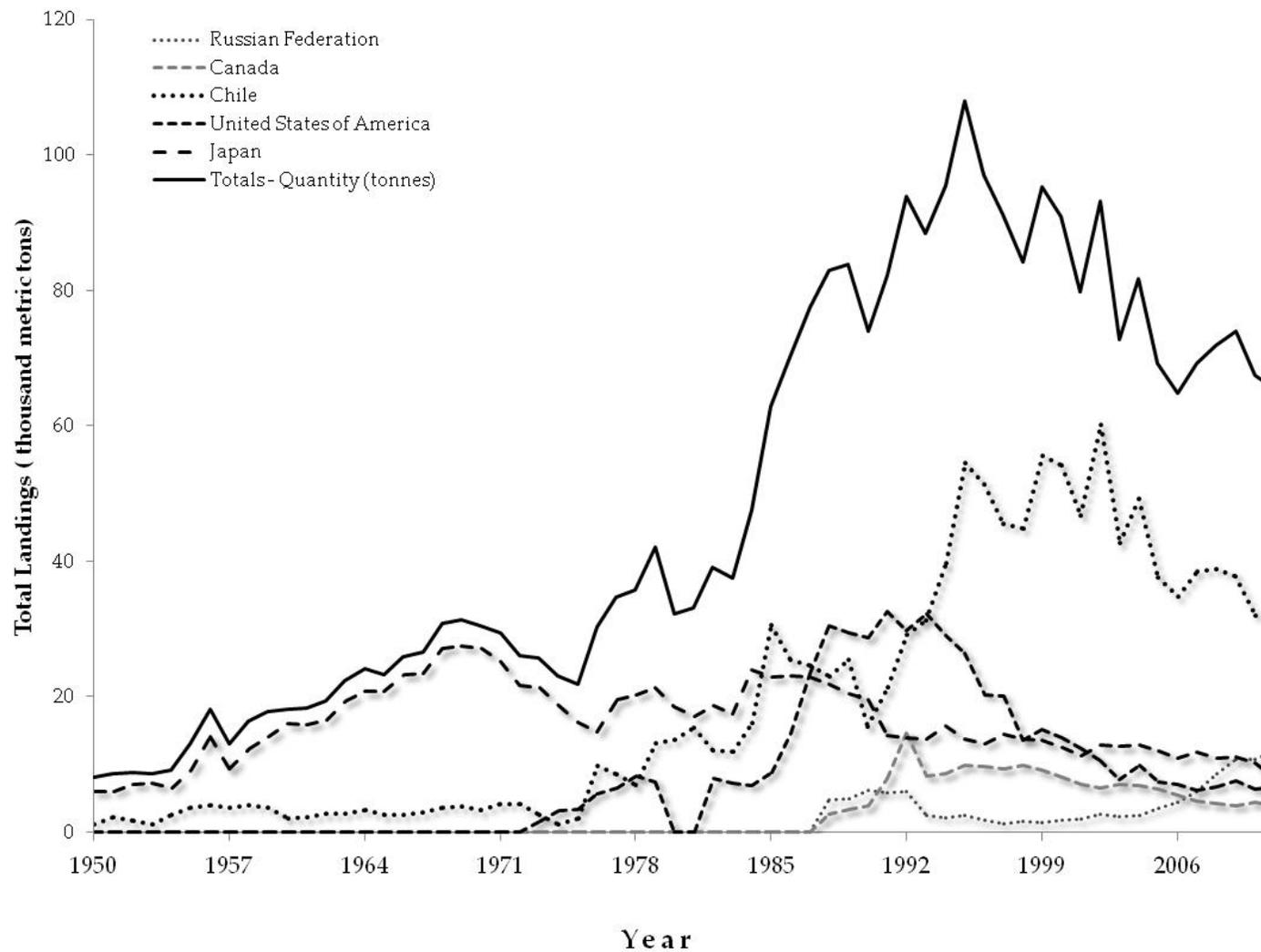


FIGURE I.2. Total sea urchin landings (thousand metric tons) from 5 major sea urchin producing countries (1950-2010).
Source: www.fao.org/fishery/statistics/global-production/en

with 27,500 metric tons, but by 2011 only 7,881 metric tons were produced (Fig. I.2). Global decline of urchin harvests was masked by the rapid expansion of the Chilean fishery in the early 1980's. When the Chilean harvests peaked in 1995 with 54,609 metric tons, they provided nearly 50% of the world's sea urchin production (Fig. I.2). In 2011 Chile still dominated sea urchin production with 31,901 metric tons, nearly 49% of total sea urchin production, but only 58% of its past peak production. In the late 1980's Russia and Canada began developing their sea urchin fisheries. Canadian production peaked at 13, 889 metric tons in 1992, but as with other countries production has been in a slow decline. In contrast, Russian sea urchin production has been growing slowly. In 2011, Russia was the second highest producer of sea urchin roe, surpassing American and Japanese production at 11, 972 metric tons (Fig. I.2).

Development of the U.S. sea urchin fishery expanded rapidly in 1973, from 58.3 metric tons landed in 1972 to 1,698 metric tons the next year. Total production peaked in 1989 and 1993, but has been on a steady decline since. By 2011 total sea urchin roe production in the United States declined to 6,664 metric tons. California and Maine are the major sea urchin producers, with landings of 5,200 and 1,091 metric tons respectively (Fig. I.3). Alaska, Washington, and Oregon also make minor contributions to domestic production. As of 2013, Japan was the biggest importer of U.S. sea urchin roe followed by South Korea and Canada (<http://www.st.nmfs.noaa.gov/st1/>).

Apart from human consumption, sea urchins also serve as animal models for embryological, cellular biological, genetic, and toxicological studies (Sluder et al. 1998). It was in sea urchin embryos that the first observations of cellular nuclear fusion were made, which was essential for the development of the theory of nuclear determination (Earnst 1997, 2011). Sea

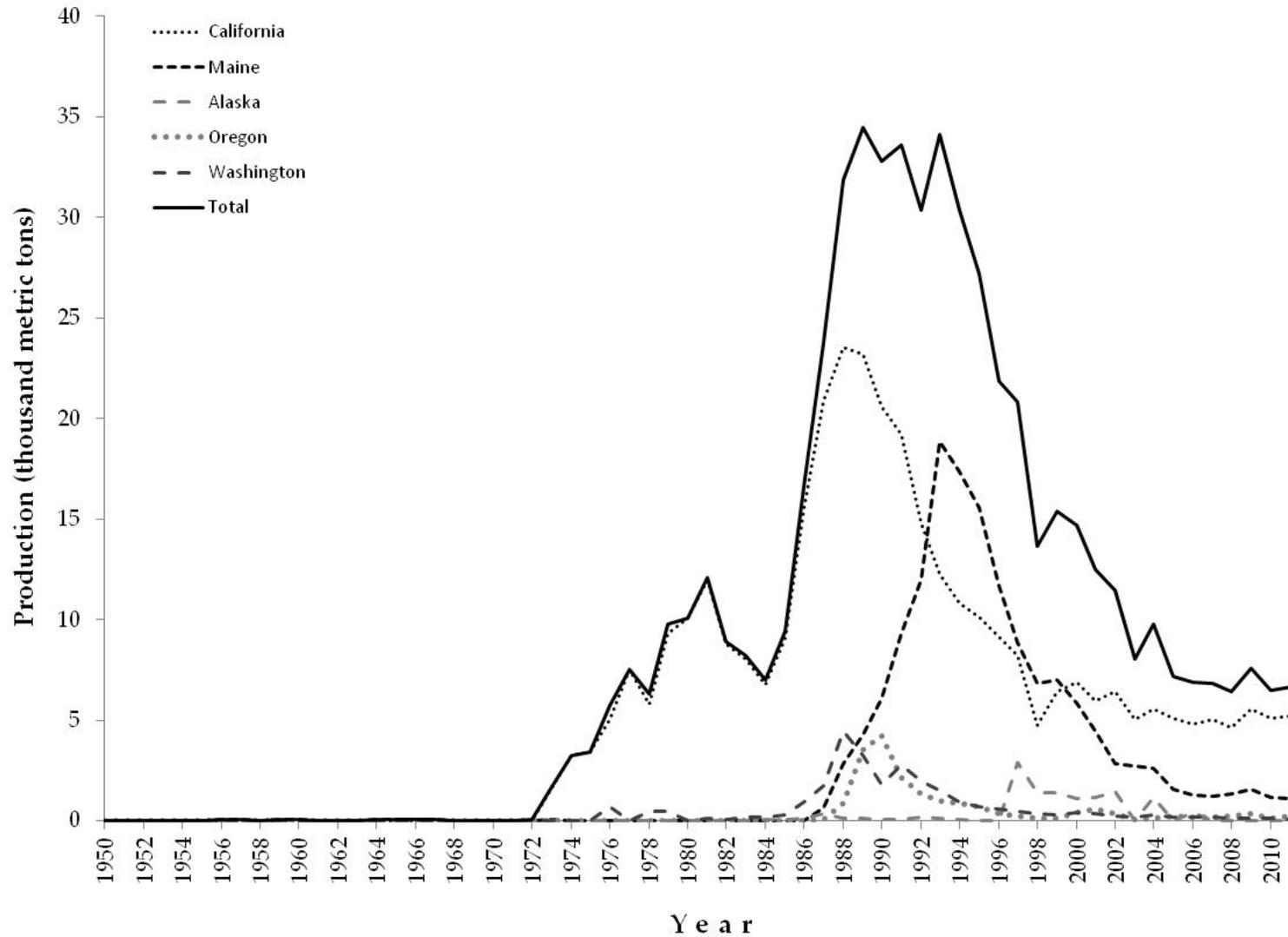


FIGURE I.3. Total U.S. sea urchin production (thousand metric tons) from 5 major urchin producing states (1950-2010).
Source: www.fao.org/fishery/statistics/global-production/en

urchins have also been used to answer crucial questions in developmental biology, embryology, and more recently genomics (Earnst 2011; Sea Urchin Genome Sequencing Consortium 2006).

Sea urchins are favored animal models because their rearing methods and artificial spawning have been well characterized (Earnst 2011; Sea Urchin Genome Sequencing Consortium 2006). Sea urchin embryos are optically clear, lack shells, have rapid and synchronous development and are easily kept in seawater (Earnst 2011; Sander and Fisher 1996; Sea Urchin Genome Sequencing Consortium 2006). For these same reasons, sea urchins are used in classrooms to illustrate basic biological and embryological concepts (Oppenheimer 1989; Epel et al. 2004). Unlike their seafood counterpart, those used in research must meet different and, in some ways, more stringent qualifications (Conn 2008). In embryological, medical, cellular biology, and genetic research, it is often preferable to have an urchin stock of known lineage and source (Conn 2008). These qualities cannot be met by wild-sourced sea urchins, thereby intensifying the need to develop better management practices and quality control criteria for sea urchin aquaculture.

Diminished wild stocks of urchins have intensified efforts at commercial culture. Successful culture of urchins requires extensive understanding of their nutritional and husbandry requirements (Lawrence and Lawrence 2003). Recent sea urchin nutritional research has focused on nutrient requirements and effects of dietary manipulation of nutrients on gonad quality (Shpigel et al. 2005; Shpigel et al. 2006; George 2001; McBride et al. 2004), somatic/gonad growth (Unuma et al. 2002; Hammer et al. 2006; Hammer et al. 2004), and survivorship (Beddingfield and McClintock 1998). *Lytechinus variegatus* requires a high-protein (~20% dry weight protein) and low-carbohydrate (~12% dry weight) diet to promote gonad growth and development (Hammer et al. 2004; Hammer et al. 2006; Cook and Kelly 2007). Inclusion of

carotenoids in prepared artificial diets is important in enhancing gonad color and quality (Shpigel 2005; 2006; McBride 2004) as well as egg and juvenile production (George 2001).

With feed costs accounting for 30-60% of operational costs in aquaculture (Naomasa et al. 2013; Rana et al. 2009; Southgate 2003), effective delivery of inexpensive but nutritionally-complete dietary components (e.g. carotenoids, protein, etc.) is of paramount importance in successful sea urchin culture. Similar to other marine aquatic species (e.g. marine penaeid shrimp), chemoattractants and feeding stimulants can increase palatability and overall consumption rate (Lawrence and Lawrence 2003; Lee and Myers 1996). For aquatic organisms fed nutritionally-replete diets, higher consumption rates could lead to improved feed conversion ratios, reduced waste, and improved water quality. Uneaten feed and metabolites of consumed feed are the major source of pollution in aquaculture systems (Lawrence and Lawrence 2003; Lawrence and Sammarco 1982). Evaluating and identifying potential chemoattractants and feeding stimulants could ultimately provide a means of formulating commercial production feeds that are nutritionally more efficient and supportive of sustainable production methodologies.

This study aims to provide a better understanding of the biology and feeding behavior of *L. variegatus*. Potential chemoattractants and specific factors (i.e. flow and nutritional state) and their effects on sea urchin feeding behavior were evaluated. In the process, a standardized and appropriate methodology for ranking and evaluating potential chemoattractants was developed for the sea urchin *L. variegatus*. This study provides a better understanding of the role of chemicals in sea urchin and animal feeding behavior.

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CHAPTER I

FLOW RATE AND ITS EFFECTS ON CONSUMPTION AND RHEOTACTIC RESPONSE IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*

Abstract

Lytechinus variegatus lack vision and relies heavily on the detection of chemical signals for successful navigation and foraging. In transit from the source, chemical signals are subject to hydrodynamic forces, such as flow velocity and turbulence. This can significantly impact an organism's efficiency and success in navigation and foraging. The effects of flow velocity and turbulence on consumption and rheotactic response were studied for the sea urchin, *Lytechinus variegatus*, in laboratory assays. Rheotactic response was defined as time of travel to an odorant over a similar distance against various current velocities. Consumption of feed was measured over a 24-hr period, while the sea urchins were subject to different current velocities. It was shown that *L. variegatus* consumed feed at higher rates with increasing flow velocity and that they are positively rheotactic, travelling faster with increasing flow rates. These findings suggest that members of this species navigate and forage more successfully in areas of high flow velocity and turbulence, similar to other sightless benthic invertebrates. The results of this study also correlate with previous observations of *L. variegatus* aggregating and feeding in areas of high flow velocity, in the wild and in culture.

Introduction

Hydrodynamic processes affect every aspect of life for aquatic organisms (Peacor and Werner 2001; Weissburg et al. 2002; Ferner and Weissburg 2005; Sato and Goshima 2007; Zimmer-Faust and Tamburri 1994; Pawlik 1992). Water velocity and turbulence can alter both the temporal and spatial presence of a chemical odorant vital for successful foraging (Weissburg 2000; Webster and Weissburg 2009; Yen et al. 1998; Koehl, 2006; Koehl et al. 2001; Hay 2009). Contingent on their inherent capacities and needs, aquatic organisms exhibit different responses to flow to optimize foraging success (Ferner and Weissburg 2005; Weissburg and Zimmer-Faust 1994; Zimmer-Faust et al. 1995; Finelli et al. 1999; Atema 1995; Moore and Grills 1999; Koehl et al. 2001; Zimmer-Faust 1989; Verheggen et al. 2007). Many organisms use flow direction as a starting point in their search, as chemical signals are typically entrained in currents. Others use flow to hide their odor from their prey or from a predator (Wisenden 2000; Kats and Dill 1998; Webster and Weissburg 2009; Ferner and Weissburg 2005). It has also been suggested that some organisms use flow to avoid competition (Ferner and Weissburg 2005).

High-velocity, turbulent flows mix and dilute chemical signals, distributing them rapidly over relatively large areas without any distinct borders (Webster and Weissburg 2009; Weissburg 2000). In contrast, low-velocity flows create odor plumes with discreet edges and concentrated chemical signals. Organisms employ different strategies to cope with these flow regimes. Fast-moving organisms with wide sensor spans are typically more successful foraging and navigating in relatively low velocity flows (Webster and Weissburg 2009; Burr 1984). Quick movement through a plume allow only for coarse perception of concentrated and instantaneous signals (Weissburg et al. 2002). Organisms like blue crabs (*Callinectes sapidus*) and American lobsters (*Homarus americanus*) rely on spatial sampling to remain within the odor plume, moving quickly between edges of the plume. In contrast, slow-moving aquatic organisms are able sample

chemical signals by integrating successive samples over time, giving them advantage in navigating in fast turbulent flows (Byron and Smee 2012; Ferner and Weissburg 2005). Several studies have investigated chemical navigation in crabs (Jackson et al. 2007; Ferner and Weissburg 2005; Weissburg and Zimmer-Faust 1994), lobsters (Atema and Gomez 1996; Koehl et al. 2001), fish (Carr et al. 1996; Adams et al. 1988), and crayfish (Moore and Grills 1999), all relatively fast-moving organisms with wide sensor arrays. Studies of chemical navigation in slow-moving animals are less common (but see Byron and Smee 2012; Ferner and Weissburg 2005; Ferner et al. 2009), and fewer still addresses the navigation strategies of echinoids (e.g. Pisut 2004).

As a group, sea urchins (e.g., *Lytechinus variegatus*) lack vision, relying heavily on olfaction for navigation and foraging. A study of tracking in *L. variegatus* suggests that turbulence and flow velocity may have limited effect on foraging success (Weissburg et al. 2002). In the wild, *L. variegatus* has been observed feeding in areas of high flow velocity (Aseltine 1982). Similarly, in culture tanks *L. variegatus* aggregate near the incoming water nozzle where the highest flow velocity can be found (Moore and McPherson 1965; personal observation).

Studying feeding and navigation in *L. variegatus* at varying flow velocities augments our understanding of foraging in sea urchins and in other slow-moving, sightless benthic organisms. From a more applied standpoint, understanding the feeding behavior of *L. variegatus* can enhance husbandry techniques used to provide products for the global seafood industry. The objectives of this research are to estimate at which flow rate rheotaxis is induced and to test the effect of flow on consumption rate. It is postulated that *L. variegatus* is positively rheotactic,

increasing its rate of movement towards the flow source with increased flow rate. It is also postulated that this species increases consumption rate with increased flow rate.

Materials and Methods

This study was conducted at the Texas A&M AgriLife Research—Shrimp Mariculture Project, Texas A&M System, Port Aransas, Texas, USA. Specimens of *L. variegatus* were supplied by the University of Alabama—Birmingham and were collected from Cape San Blas within the Port St. Joseph Peninsula State Park, FL, USA (30 °N, 85.5 °W). Upon arrival, sea urchins were maintained in a semi-recirculating system consisting of four 1.22-m diameter tanks equipped with one airstone, a central drain, an external standpipe, and an inflow water valve (Fig. 1.1). Seawater was pumped from the Corpus Christi Ship Channel and filtered through a 100- μ m filter prior to use. Water temperature, salinity, and dissolved oxygen in the tanks were monitored daily. Levels of nitrogenous metabolites (e.g., total ammonia nitrogen, nitrite) and pH were measured weekly. Water was introduced into maintenance tanks in order to create a circular current. Sea urchins were maintained on a 12h light: 12h dark photoperiod. Sea urchins were fed a standard reference diet (SRD) at ad libitum levels (Table 1.1). This diet is used routinely in all sea urchin research at the Shrimp Mariculture Project.

Rheotaxis Trial

The experimental design for this study consisted of six bulk flow-rate treatments (e.g, 0.00, 0.57, 0.95, 1.89, 3.78, and 7.57 lpm) to which sea urchins were subjected (see Table 1.4 for measured flow velocities from each treatment). A total of 10 urchins per treatment were included (mean wet weight = 71.35 ± 4.18 g) for an overall study population of 60 urchins. Treatment replicates were randomly assigned to 12 experimental tanks (15.24 cm [W] \times 45.72 cm [L] \times 10.16 cm [D]; Fig. 1.2; 1.3; 1.4). The study was divided into five, three-hour periods, with two replicates

TABLE 1.1. Standard reference diet formulation (as fed).

Ingredient	% Content
Algae,Kelp	20.000
Alginate	3.000
Calcium Carbonate	13.000
Carotein,Beta	1.000
Carotine,HiZea	0.700
Casein,Vitamin Free	4.000
Cellulose	3.000
Cholesterol	0.500
CuSO ₄ *5H ₂ O	0.020
Diatomaceous Earth	8.700
FeSO ₄ *7H ₂ O	0.120
Fish, Menhaden	5.000
KCl	2.000
MgSO ₄ *7H ₂ O	3.000
MnSO ₄ *H ₂ O	0.014
Phospholipid, 97%	4.000
PO ₄ CaH,diCaPO ₄	0.500
PO ₄ NaHexaMeta	1.000
PM.Vitamine	0.600
Soybean, 90% Protein	17.800
Squid, 79% Protein	5.000
Wheat Starch	6.920
ZnSO ₄ *7H ₂ O	0.030

of each flow treatment per period. Between each period any seawater remaining in the experimental tanks were poured out and each tank was rinsed with seawater to remove any residues (feed, spines, feces, etc.) that may have been left from the previous trial.

Water was introduced at one end (starting point; Fig. 1.3; 1.4) of each tank that was positioned with a slight incline ($\sim 0.2^\circ$) from the end point down to the starting point. This helped ensure that water flowed towards the starting point and overflowed behind it (Fig. 1.3; 1.4). Flow rates were maintained using in-line flow restrictors that let water at ~ 30 psi flow through at specified flow rates (0.57, 1.89, 3.78 lpm) by varying the diameter of the outlet port. Water expelled from the in-line flow restrictors tended to exit in a jet which can falsely increase the velocity and turbulence of the water in the tank. Six-inch long hoses with ~ 1.27 cm diameter inner lumen were attached to the flow restrictor outlet ports to dampen the water jets, allowing water to flow in a smooth stream.

At the start of each trial, one urchin was submerged at the starting point (Fig. 1.3; 1.4). The amount of time it took an urchin to travel from this point to the opposite end of the tank (end point; Fig. 1.3; 1.4) was the metric used to quantify rheotactic response. Urchins were left in the tanks for a maximum of three hours. Individual rheotactic response was recorded as minutes post-placement. Those urchins unable to reach the end point were also noted. Each urchin was removed from the tank after it reached the end point or was unable reach the end point after three hours.

Consumption Trial

This study was undertaken over five consecutive days, with one trial occurring on each of the five days, and consisted of six flow-rate treatments (e.g., 0.00, 0.57, 0.95, 1.89, 3.78, and 7.57 lpm) at a replication rate of 10 urchins per treatment ($n = 60$). On each trial day, treatments

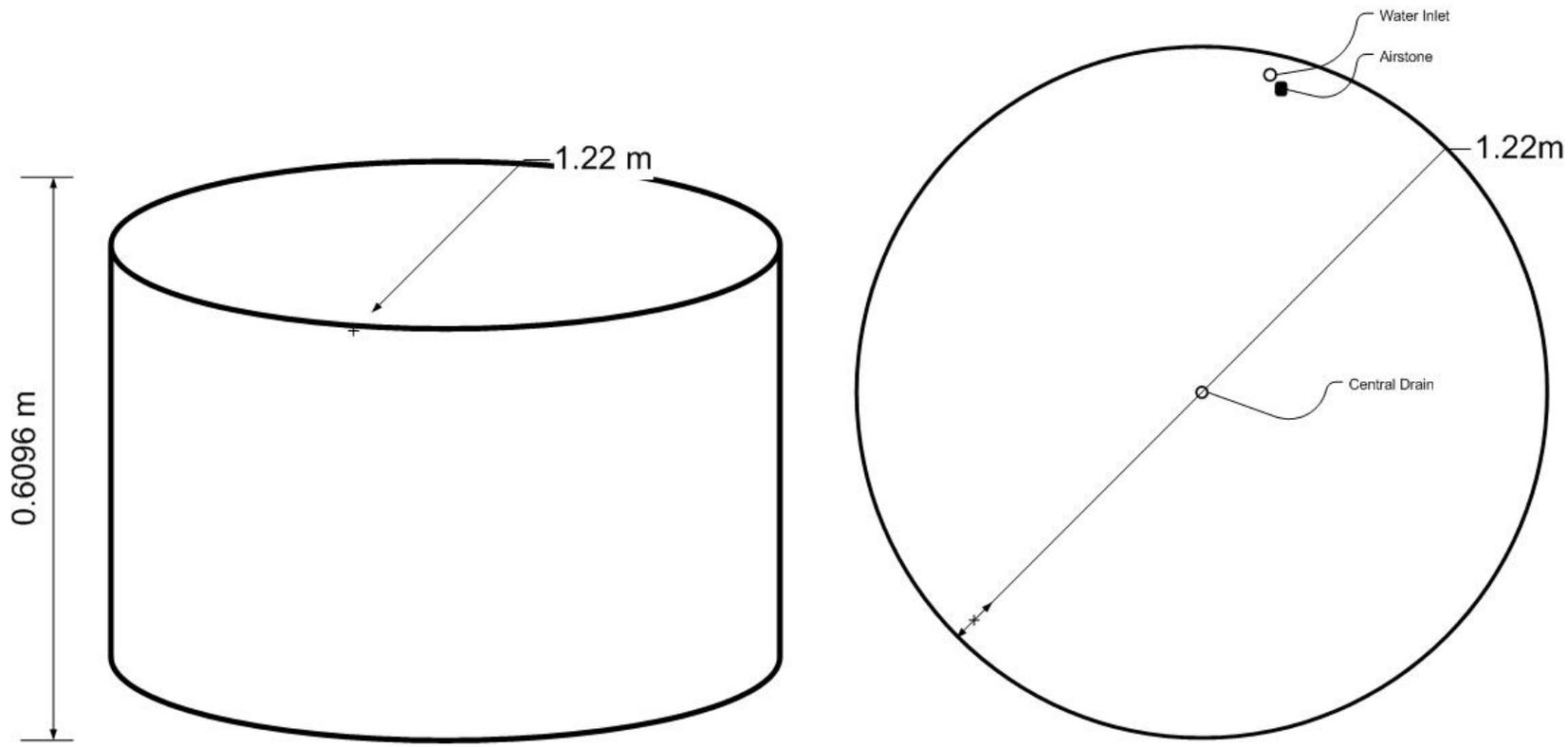


FIGURE 1.1. Holding Tanks

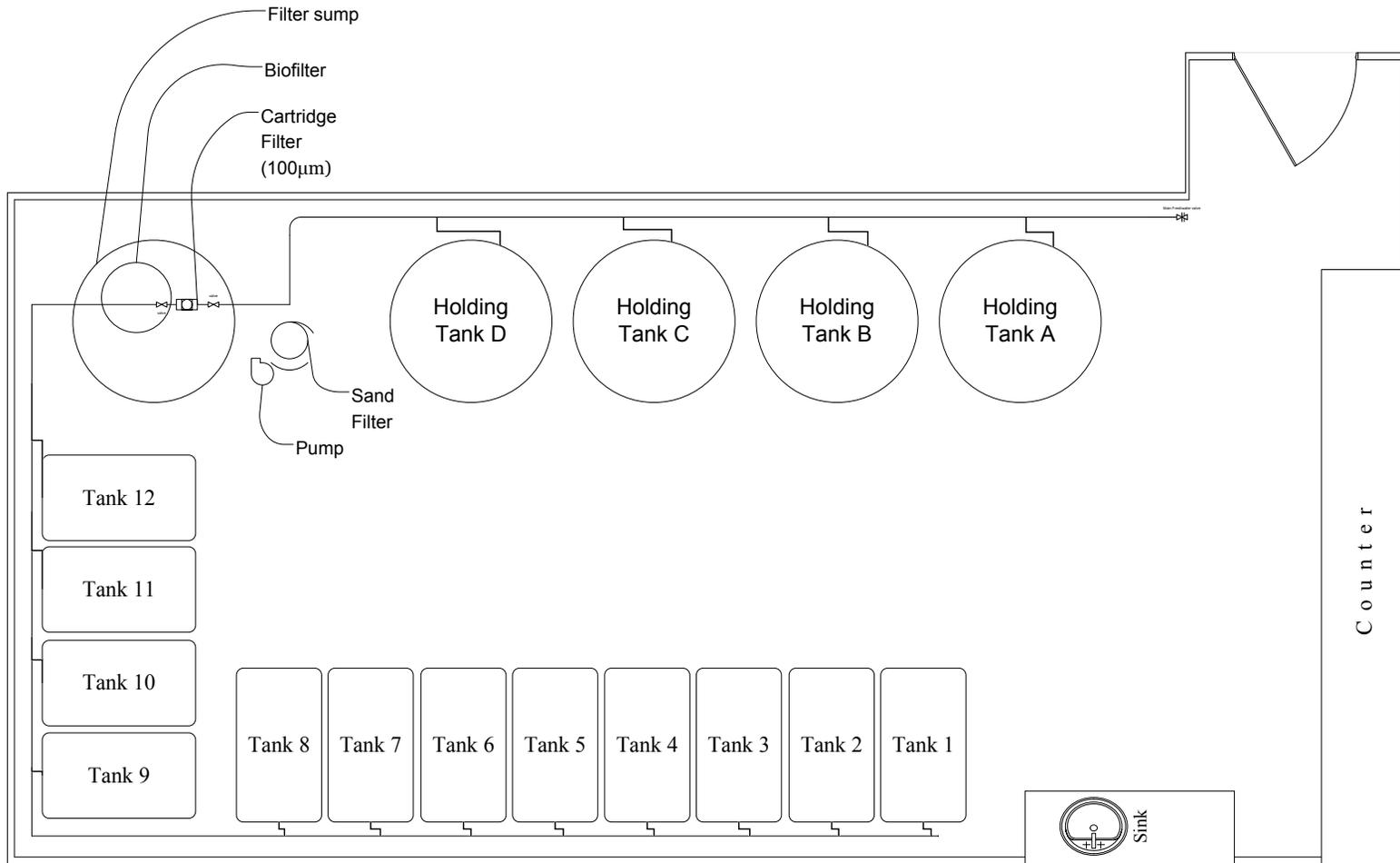


FIGURE 1.2. Urchin laboratory layout.

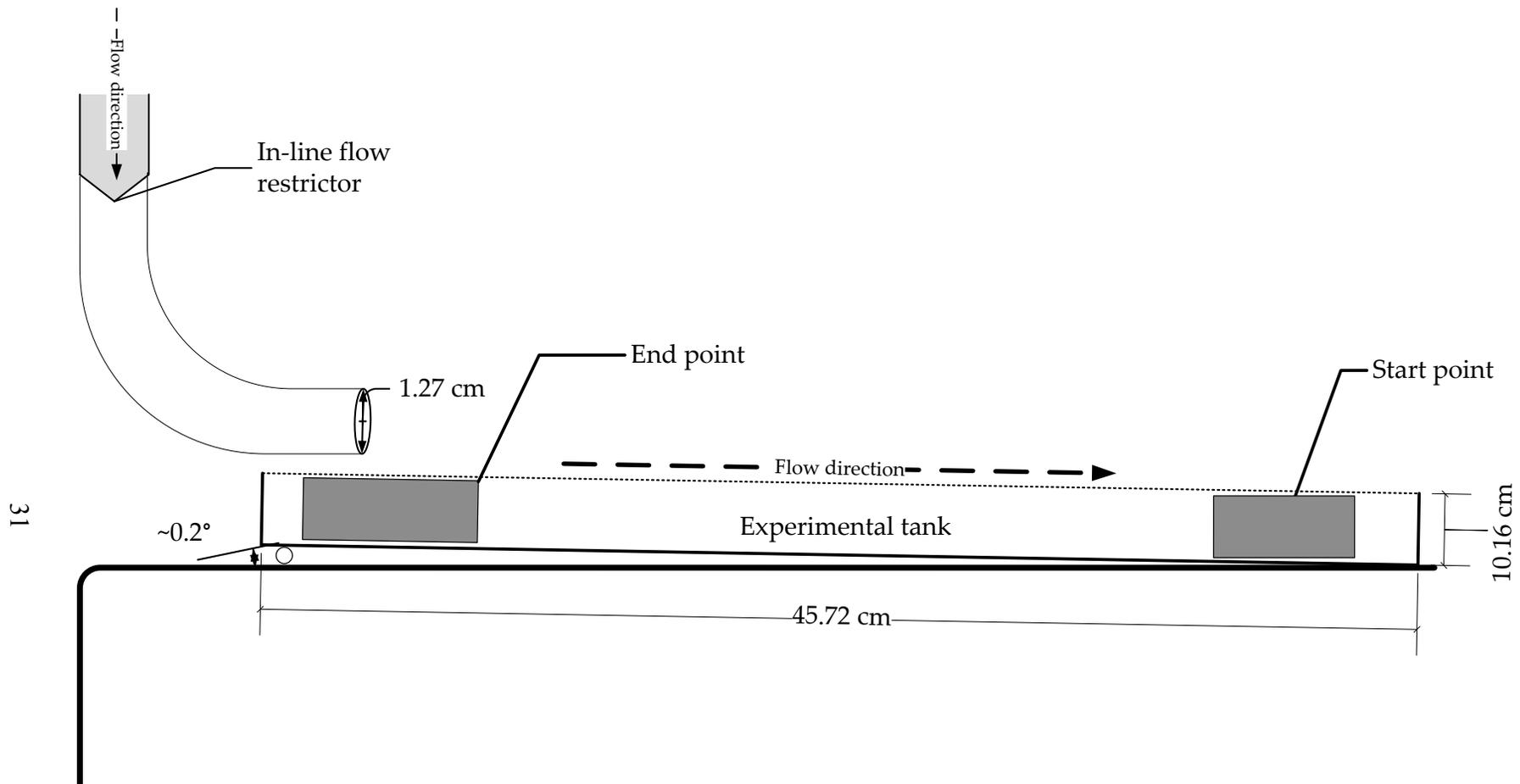


FIGURE 1.3. Rheotaxis experimental tank cross-section.

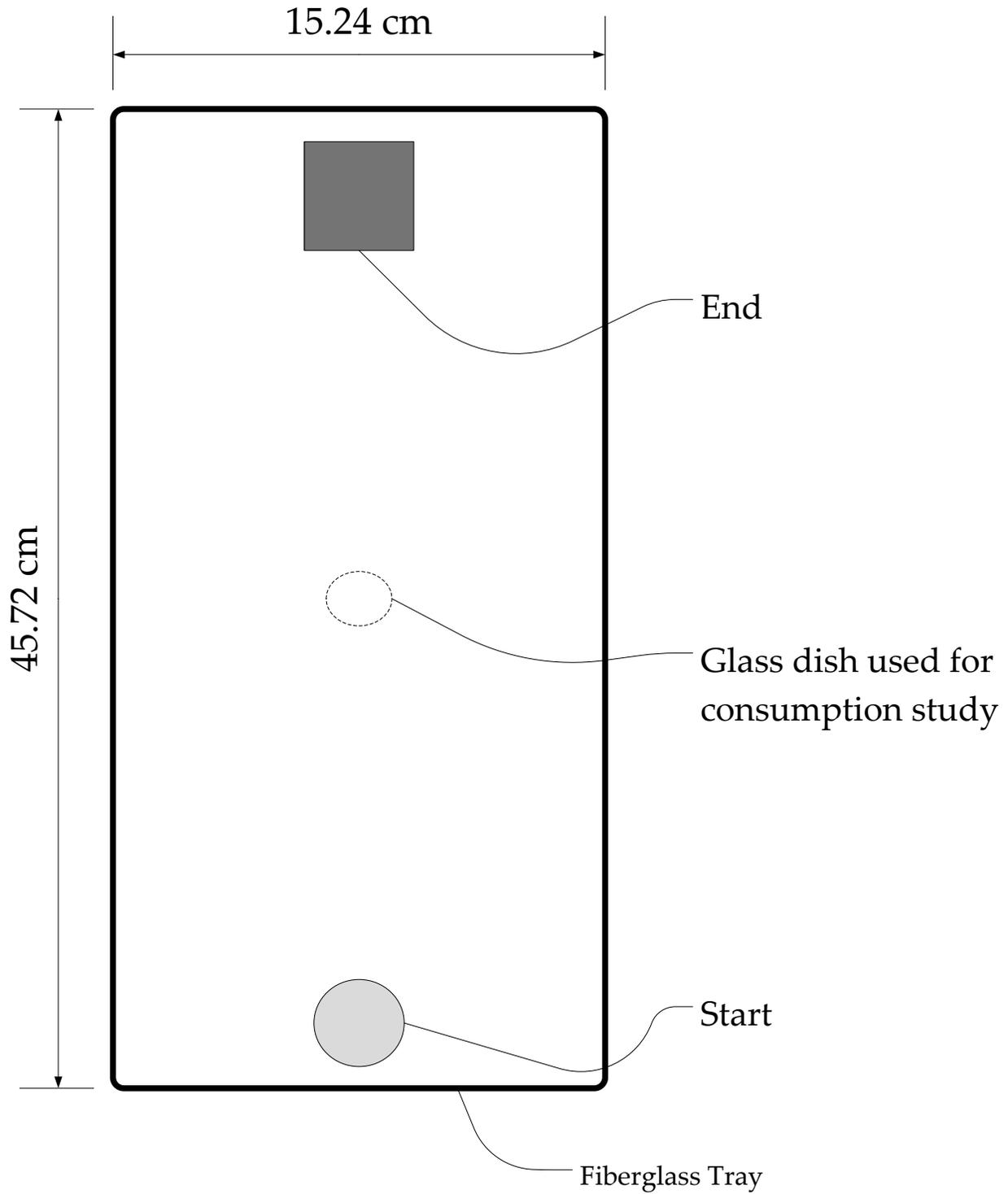


FIGURE 1.4. Rheotaxis experimental tank (top view). Box marked END is the site of water input. Sea urchins were placed on the circle marked START

were randomly assigned to tanks and tanks stocked with individual urchins (wet weight $t = 74.77 \pm 3.05$ g; $n = 60$). At the start of each trial, urchins were fed approximately 5.0 g of SRD (Table 1.1). The SRD was placed on a glass dish midpoint between the water inflow and the starting point where the urchin was initially placed (Fig. 1.3). The glass dish was used to hold the feed in place and also for quick retrieval of residual feed. After 24 hr, feed consumption was determined by removal and subsequent quantification of residual feed. Residual feed was rinsed with distilled water and dried at 60 °C in an oven for 12 hr. “Consumed” feed was determined as the difference between the dry weight of feed offered and the dry weight of feed remaining, taking into consideration losses over 24 hr not associated with feeding (i.e., leaching of dry matter into the water column). Residual seawater in experimental tanks was discarded and each tank was rinsed with seawater to remove any residues (feed, spines, feces, etc.) that may have been left from the previous trial.

Flow Characterization

Flow velocity and turbulence in each flow treatment was measured using a NortekUSA acoustic Doppler velocimeter (ADV; Vectrino model) and analyzed using Explore V software (Nortek USA, Annapolis, MD). Flow velocity was measured 5.5 cm above the tank bottom for 2 min. Turbulence was calculated using the root mean square (RMS) of the velocity time series. Measurements were taken at the starting and end points (Fig. 1.3; 1.4) and midway between the starting and end points (~22.86 cm from the water inlet hose). Velocity and turbulence (RMS) were measured in all the experimental tanks used in this study ($n=12$).

Statistical Analysis

Travel time (min.) and consumption rates (g/24hr) were analyzed using non-linear regression included in the *drc* package in R (Ritz and Streibig 2012; <http://cran.r-project.org>). A four parameter dose-response curve with the equation:

$$f(x, (b, c, d, e)) = c + \frac{d - c}{1 + \exp\{b(\log(x) - \log(e))\}}$$

was constructed to model the responses, where b is the slope, c is the lower limit, d is the upper limit and e is the Effective Dose50 (ED50 hereafter). Assumptions that have to be met for non-linear regression analysis include: 1) measurement error are normally distributed, 2) variance homogeneity, 3) independent measurements and 4) correct mean function (the equation above). Estimation of the model parameters was an iterative process automatically performed by the *drc* package using the equation above as a starter function to arrive at the best-fit model.

The flow rate that affected the shift in response for 50% of the population was estimated (Effective Dose 50; ED50 hereafter). Model estimates (e.g., slope, upper and lower limits, and 50% effective dose) were used to describe urchin responses. Dose response curve analysis is often used to describe graded responses to chemicals at different, often logarithmically spaced concentrations (Ritz and Streibig 2012). This has been applied in pharmacology, toxicology and enzymology. However, non-linear regression techniques and dose response curve analysis has also been employed to describe responses to graded variables (i.e. flow rate) other than chemicals (e.g. Vicente et al. 2006).

Results

Rheotaxis Trial

Time of travel of the sea urchin *Lytechinus variegatus* to an odorant over a similar distance was measured against various current velocities. Results indicated that at a bulk flow rate of 0.48 ± 0.27 lpm, 10% (ED10) of the population increased their speed (Table 1.2). This increase in speed plateau at 0.68 ± 0.40 lpm where 90% (ED90) of population increase their speed (Fig. 1.5; Table 1.2). Only minimal changes in bulk flow rate were needed to affect change in urchin speed, based on the slope (12.88 ± 43.55) of the fitted regression line (Table 1.2).

Consumption Trial

Consumption rate increased directly with flow rate (Fig. 1.6). The ED50 was estimated 0.63 ± 0.08 lpm (Table 1.3). Response to increased bulk flow rates was gradual (slope= -1.66 ± 0.41 ; Fig. 1.6), but the ED10 was only 0.17 ± 0.06 lpm. This means that minimal increase in flow rate ($\sim 0.17 \pm 0.06$ lpm) could increase consumption in 10% of the population tested. Maximum consumption rate was achieved (3.49 ± 0.11 g/24 hr) when urchins were subjected to 2.36 ± 0.81 lpm (Table 1.3).

Flow Characterization

Flow velocity was higher at the end point where water was introduced to the system (Table 1.4). Flow velocity did not increase with increases in bulk flow rate. Increase in flow rate only had minimal effect on flow velocity. In contrast, turbulence (RMS) increased with increasing bulk flow rate (Table 1.4). Turbulence was highest at the end point and gradually decreased towards the starting point.

TABLE 1.2. Rheotaxis non-linear regression model parameter estimates with associated Standard Error (S.E.).

	Estimate	Standard Error
Slope	12.88	43.55
Upper Limit (min.)	64.35	5.55
Lower Limit (min.)	19.57	2.83
ED10 (lpm)	0.48	0.27
ED50 (lpm)	0.57	0.03
ED90 (lpm)	0.68	0.40

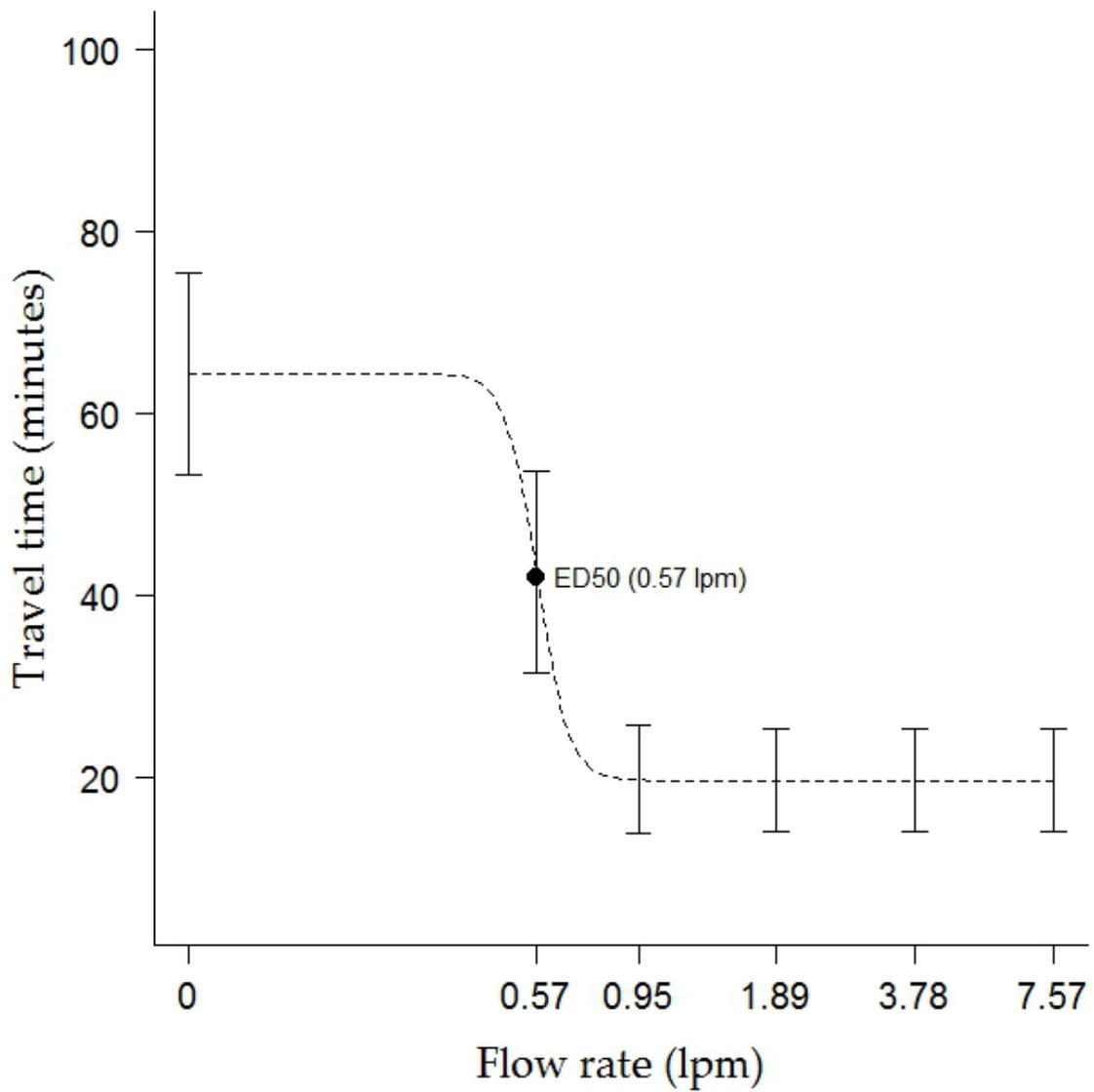


FIGURE 1.5. Mean sea urchin travel time (minutes) at different flow rates (lpm) with estimated ED50. (Error Bars=S.E.)

TABLE 1.3. Consumption non-linear regression model parameter estimates with associated Standard Error (S.E.).

	Estimate	Standard Error
Slope	-1.66	0.41
Upper Limit (g)	3.49	0.11
Lower Limit (g)	1.34	0.10
ED10 (lpm)	0.17	0.06
ED50 (lpm)	0.63	0.08
ED90 (lpm)	2.36	0.81

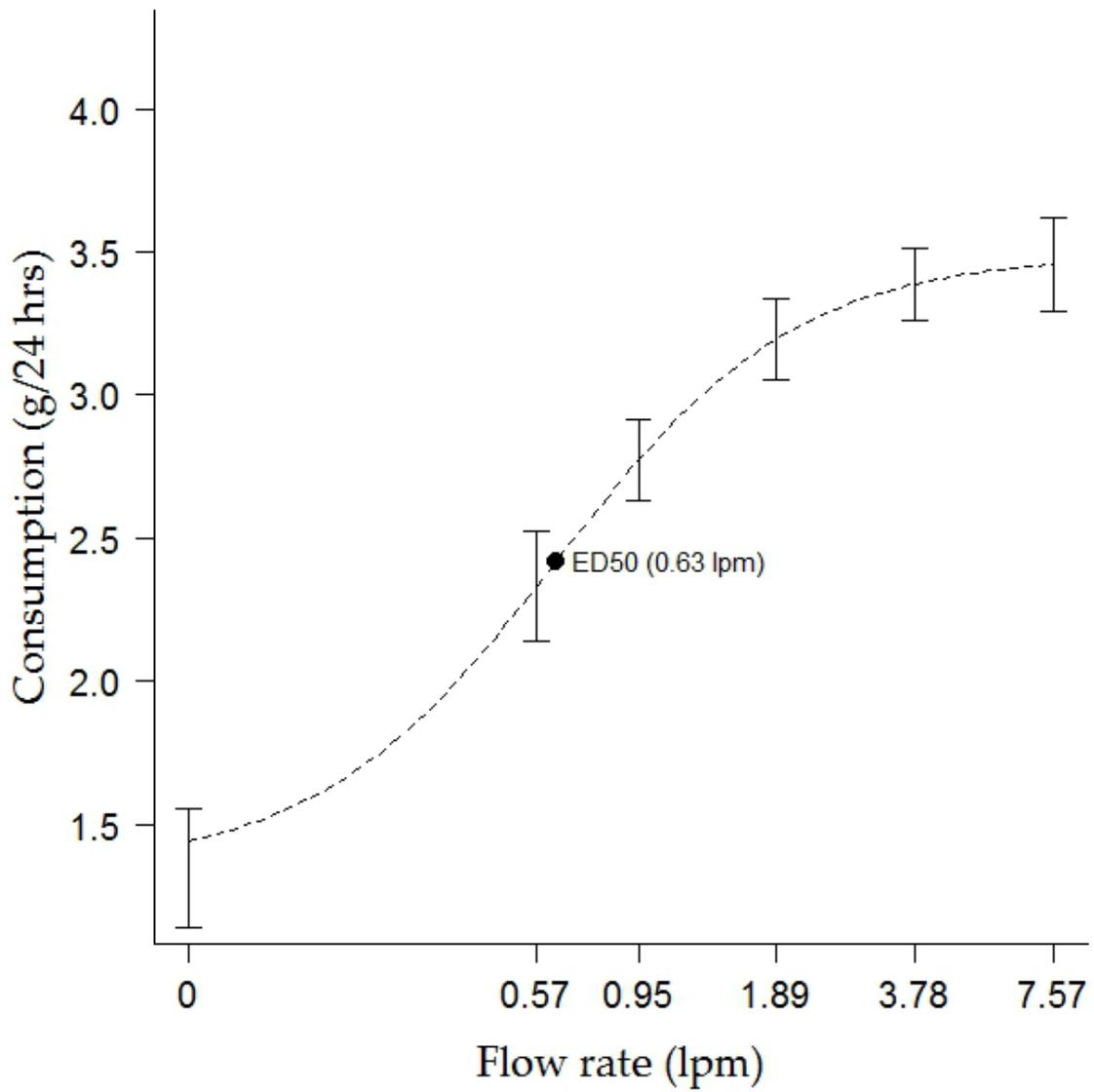


FIGURE 1.6. Mean sea urchin consumption (g/24hrs) at different flow rates (lpm) with estimated ED50. (Error bars=S.E.)

TABLE 1.4. Flow characteristics values. Values were calculated from acoustic Doppler velocimeter measurements.

	Flow Velocity (cm/s)			RMS (cm/s)		
	Start	Middle	End	Start	Middle	End
0 lpm	1.28×10^{-4}	1.58×10^{-4}	1.52×10^{-4}	2.89×10^{-3}	3.07×10^{-3}	1.25×10^{-3}
0.57 lpm	1.56×10^{-4}	7.51×10^{-4}	1.79×10^{-3}	2.34×10^{-3}	3.73×10^{-3}	9.46×10^{-3}
0.95 lpm	2.19×10^{-3}	1.75×10^{-4}	3.74×10^{-4}	4.90×10^{-3}	3.89×10^{-3}	7.31×10^{-3}
1.89 lpm	7.26×10^{-4}	4.09×10^{-4}	5.96×10^{-4}	3.71×10^{-3}	4.70×10^{-3}	7.75×10^{-3}
3.78 lpm	2.28×10^{-3}	1.73×10^{-3}	5.07×10^{-4}	8.77×10^{-3}	9.60×10^{-3}	1.00×10^{-2}
7.57 lpm	1.52×10^{-3}	3.00×10^{-3}	5.89×10^{-3}	6.14×10^{-3}	1.39×10^{-2}	4.27×10^{-2}

Discussion

Lytechinus variegatus increased rate of feed consumption as flow rate increased (Fig.1.6) and were positively rheotactic (Fig. 1.5). This species has been observed foraging in areas with high flow velocity (Moore and McPherson 1965; Beddingfield and McClintock 2000). In holding tanks, *L. variegatus* often aggregate close to the water inlet where water velocities are greater and this occurs even when water quality is degraded or dissolved oxygen is depleted (Moore and McPherson 1965). In the field, the tendency to forage in areas of relatively high water flow velocity could present increased potential for encounters with drifting food items and avoidance of competitors and predators. The knobbed whelk, *Busycon carica*, also a slow-moving benthic organism, has demonstrated superior foraging success over that of its competitor, the blue crab (*Callinectes sapidus*) in fast, turbulent flows (Ferner and Weissburg 2005; Weissburg and Zimmer-Faust 1993). In the current study, increase in bulk flow rate did not have a concomitant increase in flow velocity; however, turbulence (RMS) increased directly with flow rate (Table 1.4). It may be that *L. variegatus* is attracted to areas of high turbulence and not necessarily to areas of increased flow velocity. Improved foraging success in turbulent flows appears to provide the knobbed whelk a separate niche for foraging for the same prey as the blue crab, thereby reducing competition (Ferner and Weissburg 2005). The southern oyster drill *Stramonita haemastoma* forages effectively in both flow and fast flows (Byron and Smee 2012).

Homogenization of the odor plume in high velocity flows diminishes the presence and intensity of odor filaments (Weissburg and Zimmer-Faust 1993; Ferner and Weissburg 2005). Two strategies have been seen employed by organisms to navigate in turbulent high velocity flows (Webster and Weissburg 2009; Ferner and Weissburg 2005; Weissburg 2000). The relatively fast-moving crab adjusts its position within the plume by moving from one edge to the other, integrating samples from its laterally separated sensors (Jackson et al. 2007; Weissburg

and Zimmer-Faust 1994). Slower moving organisms, unable to quickly move between the edges of a plume are predisposed to integrative sampling over a period of time to estimate mean concentration of an evanescent and diluted signal (Webster and Weissburg 2009; Ferner and Weissburg 2005; Weissburg and Zimmer-Faust 1993). Temporal integration allows foragers to discriminate low concentration signals, even in high flow and turbulent environments (Webster and Weissburg 2009; Burr 1984). In flume studies, *L. variegatus* has been shown to follow a relatively straight path towards the odor source (Pisut 2004; personal observation), similar to the slow moving gastropod, *Busycon carica* (Ferner and Weissburg 2005). This could indicate that *L. variegatus*, due to its slow movement and limited capacity for spatial sampling, might intrinsically forage with more success in high-velocity flows, hence their persistence in such environments.

Organisms inhabiting turbulent environments utilize flow to provide a general direction towards an odorant (Weissburg and Zimmer-Faust 1993; 1994). This could explain the rheotactic behavior of *L. variegatus* despite the lack of an odor source (Fig. 1.5). Aquatic animals are able to narrow their search area in the absence of odor by moving across currents when they initially search for or lose track of odor plumes (Weissburg and Zimmer-Faust 1993; 1994; Finelli et al. 1999).

Although the affinity of *L. variegatus* for high-flow-velocity currents in its natural habitat has certain ecological benefits, it allows urchins to increase their consumption rate; which could have negative implications for its commercial production via aquaculture. Previous studies with aquaculture animals have shown that increased rate of consumption is positively related to higher percent weight gain per unit time (Christiansen and Siikavuopio 2007; James and Siikavuopio 2012; Xia et al. 2007). However, it is likely that the stability of most aquaculture feeds would be

negatively affected (e.g., increased dry matter loss due to scouring or nutrient leaching) if placed into a high water velocity situation (Lawrence and Lawrence 2003). This issue could be resolved by inclusion of higher levels of binders or nutrient-containing ingredients imparting higher water stability to pellets as well as feed chemoattractants. Ultimately, these measures would result in an increase in feed ingredient and, possibly, manufacturing costs (Lawrence and Lawrence 2003; Lee and Myers 1996). In addition to the aforementioned issues, culture system designs might have to be modified to compensate for the huddling behavior of *L. variegatus* in areas of high flow velocity. However, increased consumption rates resulting from higher flow rates could offset higher energy costs for maintaining such flows.

Foraging represents a necessary activity in slow-moving aquatic animals, whose success is determined by avoidance of predators and reduction of competition (Tomba et al. 2001; Hay 2009; Weissburg 2000; Webster and Weissburg 2009). This study showed that *L. variegatus* consumed feed at higher rates and was positively rheotactic in high-velocity currents. Additional work needs to be undertaken to ascertain whether the relationships between flow rate and feed consumption/rheotaxis derived from this study exist in the natural environment.

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CHAPTER II

FOOD LIMITATION AND ITS EFFECTS ON CHEMOTAXIS AND CONSUMPTION IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*

Abstract

Food is a limited resource in most habitats and all organisms encounter some form of food limitation in their lifetime. This study evaluated the effect of food limitation and intermittent feeding on chemotactic response and consumption rate in the sea urchin *L. variegatus*. Urchins of similar weight ($41.22 \pm 2.77\text{g}$; $n = 32$) were randomly placed into individual cages inside a larger tank (76cm (L) \times 46cm (W) \times 31cm (D)) at a water depth of 13 cm. Each urchin was randomly assigned one of four treatments: 1) ad libitum feed ration, daily; 2) ad libitum feed ration, every two days; 3) ad libitum feed ration, every four days; and 4) no feed for duration of trial. Every four days, chemotaxis was determined by measuring travel time of each urchin in response to the standard reference diet (SRD) as a chemical odorant. For urchins in treatments in which feed was offered (Treatments 1, 2, 3), consumption was measured every four days. To measure consumption, a known amount of feed was offered to each urchin. Uneaten feed was then collected after 24 h. Individuals that were fed once daily traveled toward the chemoattractant significantly faster ($p < 0.001$) than starved individuals, but consumed significantly less feed ($p < 0.001$) than individuals in other treatments in which feed was proffered. These results indicate that chemotaxis and duration of starvation are inversely related suggesting that *L. variegatus* decreases physical activity during periods of prolonged food limitation, but consumes larger amounts of food when it becomes available again. This compensatory feeding strategy could allow for energy and resource conservation when food is limited. This capacity increase starvation tolerance and chances of encountering food while simultaneously affording quick recovery of lost resources when they become available.

Introduction

Periods of food limitation are commonly encountered by animals largely due to the variability of food abundance and quality over time and space (Sommer 1984). Plankton populations are controlled by limited resources (Purcell et al. 1994; Finkel et al. 2004), many fish overwinter with little or no food (Biro et al. 2004) and sea urchins are often found in denuded seagrass meadows (Aseltine 1984). In order to survive, animals must use internal energy reserves to fuel basal metabolism, physical activity, growth and reproduction when food is not available. In addition, they exhibit adaptive behavioral and physiological responses to food deprivation. However, overall tolerance to food limitation depends on the ability to store energy and control its allocation during extreme bouts of starvation (Sommer 1984; Wang et al. 2006).

The physiological response of sea urchins to prolonged periods of food deprivation is characterized as decreased metabolic activity with respect to gonad production and somatic growth (Guillou et al. 1999; James and Siikavuopio 2012). With continued food limitation, *L. variegatus* will utilize organic reserves within the body for energy until expended and resulting in either death or recuperation once food becomes available again (Lares and Pomory 1998). These responses help conserve energy so that vital physical activities such as foraging or avoiding predators can be maintained and ensures that digestive and metabolic processes can resume when food becomes available (Lawrence et al. 2003; Wang et al. 2006).

Decreasing physical activity may contribute more to curtailing energy expenditure than reduced metabolic rate (Wang et al. 2006). During food deprivation animals can either actively search for food and expend energy or decrease physical activity to conserve energy. The use of these strategies depends on the foraging method of the animal, the causes of food limitation and other factors in its life history (Wang et al. 2006; Sommer 1984). In general, active predators and grazers are more likely to actively search for food when not available, whereas other predators

(e.g., snakes and alligators) have been shown to reduce activity (Wang et al. 2006; Sommer 1984).

Behavioral response to prolonged food limitation can be variable. For example, captive rats (Koubi et al. 1991; Scalafani and Rendel 1978) and emperor penguins (Robin et al. 1998) reduce physical activity during the initial stages of food deprivation. In the latter stages of starvation, a period of hyperactivity occurs as lipid stores become depleted. It is at this point that the need for food becomes acute. In contrast, some fishes decrease physical activity in later stages of starvation as an attempt to conserve energy until presented with the possibility of food (Mendez and Wieser 1993; Van Dijk et al. 2002).

Studies on the behavioral response of sea urchins are limited. Compensatory feeding has been observed in the sea urchin, *Paracentrotus lividus* (McCarron et al. 2009) and *Strongylocentrotus droebachiensis* (James and Siikavuopio 2012). In these instances, when food was available, sea urchins consumed more, but notably if the food source was nutritionally-deficient or limited. The present study characterizes the behavioral response of sea urchins at the onset of starvation, a subject that has not been adequately explored.

This study attempts to identify the behavioral strategy that the sea urchin *Lytechinus variegatus* employs during periods of food limitation. Understanding how starvation influences chemotaxis and consumption rate in this species could better explain local distribution and feeding patterns. This could also be useful in optimizing sea urchin husbandry in aquaculture. Given the life history and foraging mode of *L. variegatus*, it is posited that *L. variegatus* will decrease chemotactic activity with prolonged food limitation and increase feed consumption rate when fed intermittently.

Materials and Methods

This study was conducted at the Texas A&M AgriLife Research—Shrimp Mariculture Project, Texas A&M System, Port Aransas, Texas, USA. Specimens of *L. variegatus* were supplied by the University of Alabama—Birmingham and were collected from Cape San Blas within the Port St. Joseph Peninsula State Park, FL, USA (30 °N, 85.5 °W). After receipt, sea urchins were maintained in a semi-recirculating system consisting of four 1.22-m diameter tanks equipped with one airstone, a central drain, an external standpipe, and an inflow water valve (see Fig. 1.1; 1.2). Seawater was pumped from the Corpus Christi Ship Channel and filtered through a 100- μ m filter prior to use. Water temperature, salinity, and dissolved oxygen in the tanks were monitored daily. Levels of nitrogenous metabolites (e.g., total ammonia nitrogen, nitrite) and pH were determined weekly. Water was introduced into maintenance tanks in order to create a circular current. Sea urchins were maintained on a 12h light: 12h dark photoperiod and fed a standard reference diet (SRD) at ad libitum levels (see Table 1.1). This diet is used routinely in all sea urchin research at the Shrimp Mariculture Project.

Urchins of similar weight (41.13 ± 2.67 g; n=75) were randomly placed in individual cages (Fig. 2.1a) inside a larger tank (76cm (L) \times 46cm (W) \times 31cm (D); Fig. 2.1b) containing a water depth of 13 cm. Sea urchins were allowed to acclimate to experimental conditions for five days prior to the start of the study. During the acclimation period urchins were fed the standard reference diet (SRD; Table 1.1) ad libitum daily. Each urchin was then randomly assigned one of four treatments: 1) ad libitum feed ration, daily; 2) ad libitum feed ration, every two days; 3) ad libitum feed ration, every four days; and 4) no feed for the duration of the trial (20 days).

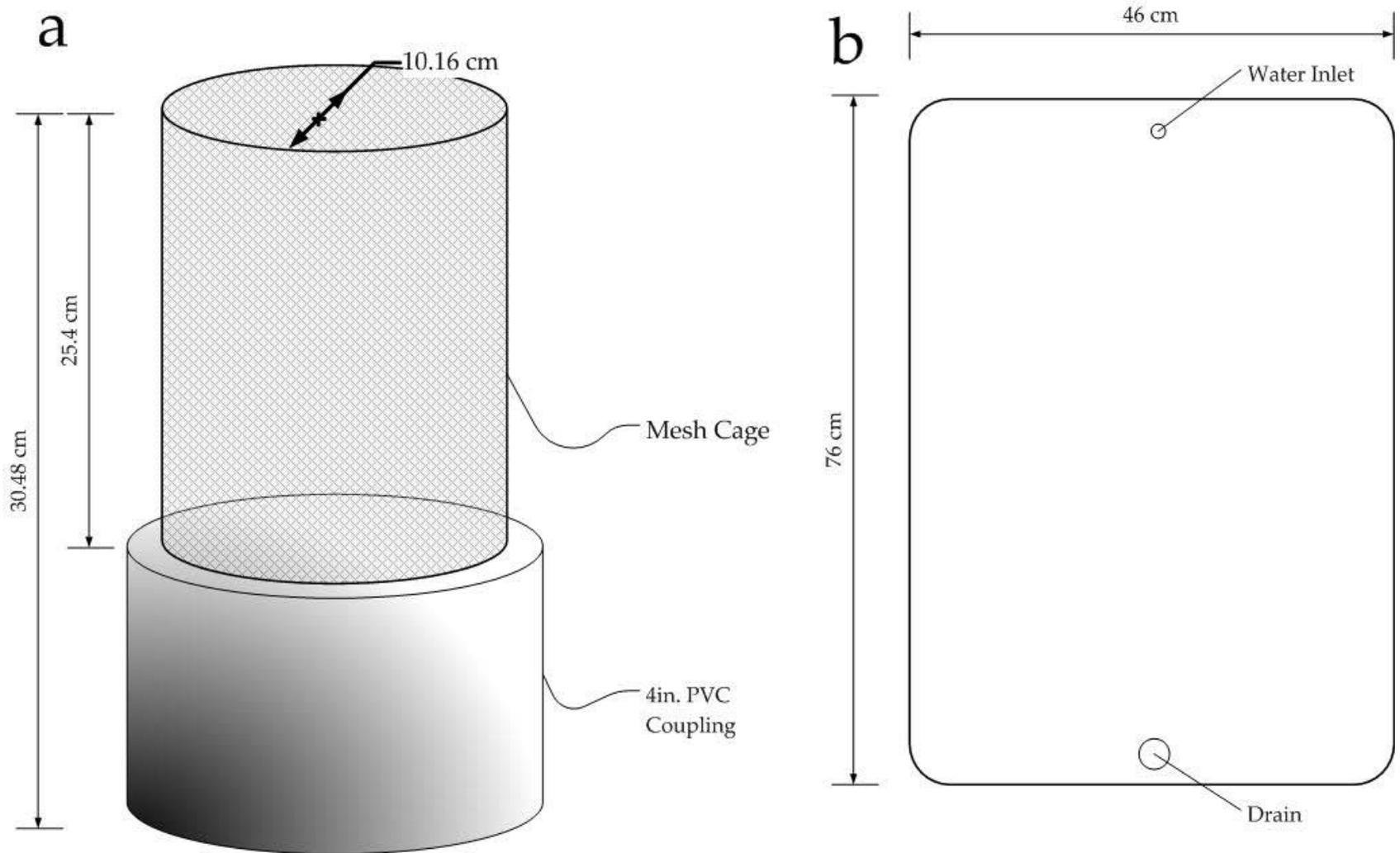


FIGURE 2.1. a) Cages used to contain individual urchins; b) Tank used to hold cages with urchins.

Chemotaxis Trial

Every four days, chemotaxis response was determined by measuring the travel time of 32 urchins (8 urchins/ treatment; n=32) toward ~1 g of the chemical attractant (SRD; Table 1.1). This part of the study was performed from 09:00 to 14:00 in shallow tanks (15.24 cm (W) × 45.72 cm (L) × 10.16 cm (D); Fig. 1.3; 1.4). Seawater used for this study was obtained from the same recirculating system used for the maintenance of the sea urchins. Water temperature was maintained at 22.4 ± 1.2 °C and dissolved oxygen at 8.62 ± 0.7 mg/L. Salinity averaged 35 ± 2 ppt. Ammonia, nitrite, and nitrate were maintained at nominal levels for sea urchin culture. Water flow rate in the tanks was controlled using flow restrictors set at ~0.57 lpm (Fig. 1.3; 1.4).

At the start of each trial, one urchin was submerged on the opposite side of the tank from the input of seawater. This location also served as the starting point of the trial (Fig. 1.3; 1.4). The amount of time required for an urchin to travel from this starting point to the opposite end of the tank (end point; Fig. 1.3; 1.4) was the metric used to quantify rheotactic response. Urchins were left in the tanks for a maximum of three hr. Individual rheotactic responses were recorded as minutes post-placement. Those urchins unable to reach the end point were also noted. Each urchin was removed from the tank after it reached the end point or was unable reach the end point after three hr. A new trial was started immediately afterwards with a new sea urchin. Each tank was rinsed between each trial to remove any residues (feed, spines, feces, etc.) that may have been left from the previous trial.

Consumption Trial

Consumption was measured only for those urchins offered feed during the trial (those fed ad libitum daily, ad libitum every 2 days and those fed ad libitum every 4 days). Every four days, a known amount of feed was proffered to each urchin according the assigned treatment.

Uneaten feed was collected 24 hr post-feeding. Collected feed was rinsed with distilled water and dried at 60 °C in an oven for 12 hours. Dry feed consumption was determined as:

$feed\ given\ (g) - feed\ remaining\ (g)$. The approximate moisture content of the feed and the loss of feed into the water were determined using controls and taken into account.

Statistical Analyses

Chemotactic response and consumption were analyzed using Repeated Measures ANOVA with SPSS 16.0 statistical software (IBM SPSS software, <http://www.spss.com>). Differences in responses between treatments were compared using Tukey's HSD.

Results

Chemotaxis Trial

The travel time of urchins over a fixed distance was measured for sea urchins subjected to different feeding regimes. Those urchins fed every four days and starved for 20 days travelled slower as the 20-day study progressed; however, this decrease in speed was more pronounced in starved individuals (Fig. 2.2). Sea urchins fed every four days travelled significantly slower than urchins fed daily ($p=0.019$; Table 2.1) but still significantly faster than starved individuals ($p<0.001$). Average travel time for starved sea urchins was significantly slower over the 20 day trial period ($p<0.001$; Fig. 2.3) compared to individuals in all other treatments. Fed urchins travelled significantly faster than all other treatments (Table 2.1; Fig. 2.3).

Consumption Trial

Consumption of feed by sea urchins fed at an ad libitum rate was measured at different frequencies (e.g., fed every two days, every four days and daily). Data analysis revealed that the assumption of sphericity of variance for repeated measures ANOVA was not met (Mauchly's

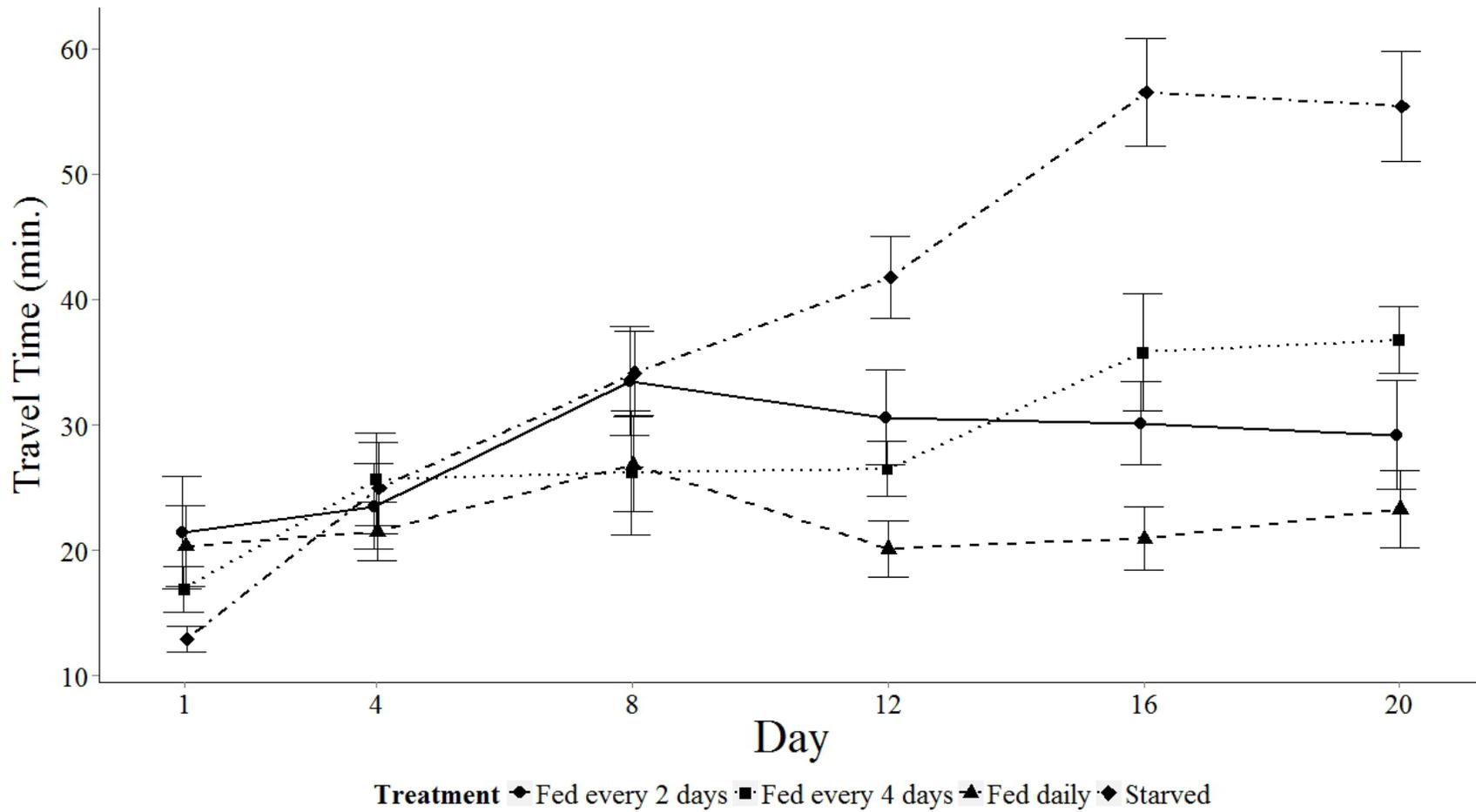


FIGURE 2.2. Mean travel time (minutes) of individuals fed ad libitum daily, every 2 days, every 4 days and starved for 20 days (Error bars=S.E.).

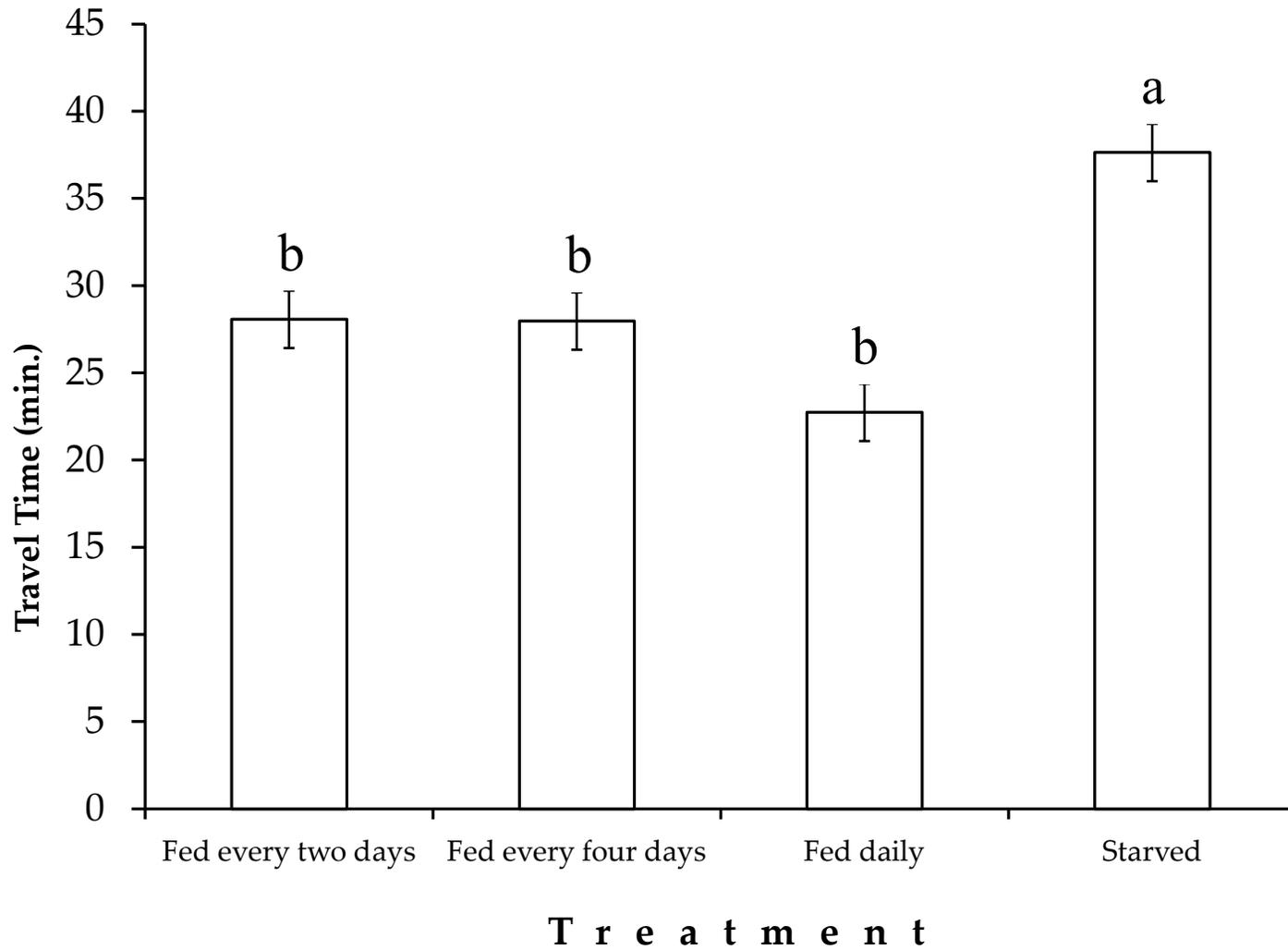


FIGURE 2.3. Mean sea urchin travel time (minutes) for each treatment over the 20 day trial period (Error Bars=S.E.). Letters above each treatment represent significant differences (α 0.05). Treatments that share the same letter are not significantly different.

TABLE 2.1. Pairwise comparison of travel time between each treatment (Fed every two days, Fed every four days, Fed daily and starved for 20 days). The mean difference is significant at α 0.05.

Treatment	Treatment	Mean Difference	Std. Error	Sig.
Fed every 2 days	Fed every 4 days	0.097	2.328	0.967
	Fed daily	5.891	2.328	0.017
	Starved	-9.567	2.328	0.000
Fed every 4 days	Fed every 2 days	-0.097	2.328	0.967
	Fed daily	5.794	2.328	0.019
	Starved	-9.664	2.328	0.000
Fed daily	Fed every 2 days	-5.891	2.328	0.017
	Fed every 4 days	-5.794	2.328	0.019
	Starved	-15.458	2.328	0.000
Starved	Fed every 2 days	9.567	2.328	0.000
	Fed every 4 days	9.664	2.328	0.000
	Fed daily	15.458	2.328	0.000

TABLE 2.2. Tests of within-subjects effects for consumption rates of urchins fed at different intervals (every 2 days, every 4 days and daily) over 20 days. The mean difference is significant at α 0.05.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
day	Huynh-Feldt	0.154	4.854	0.032	8.756	0.000
day * treatment	Huynh-Feldt	0.406	9.707	0.042	11.568	0.000
Error(day)	Huynh-Feldt	1.262	349.467	0.004		

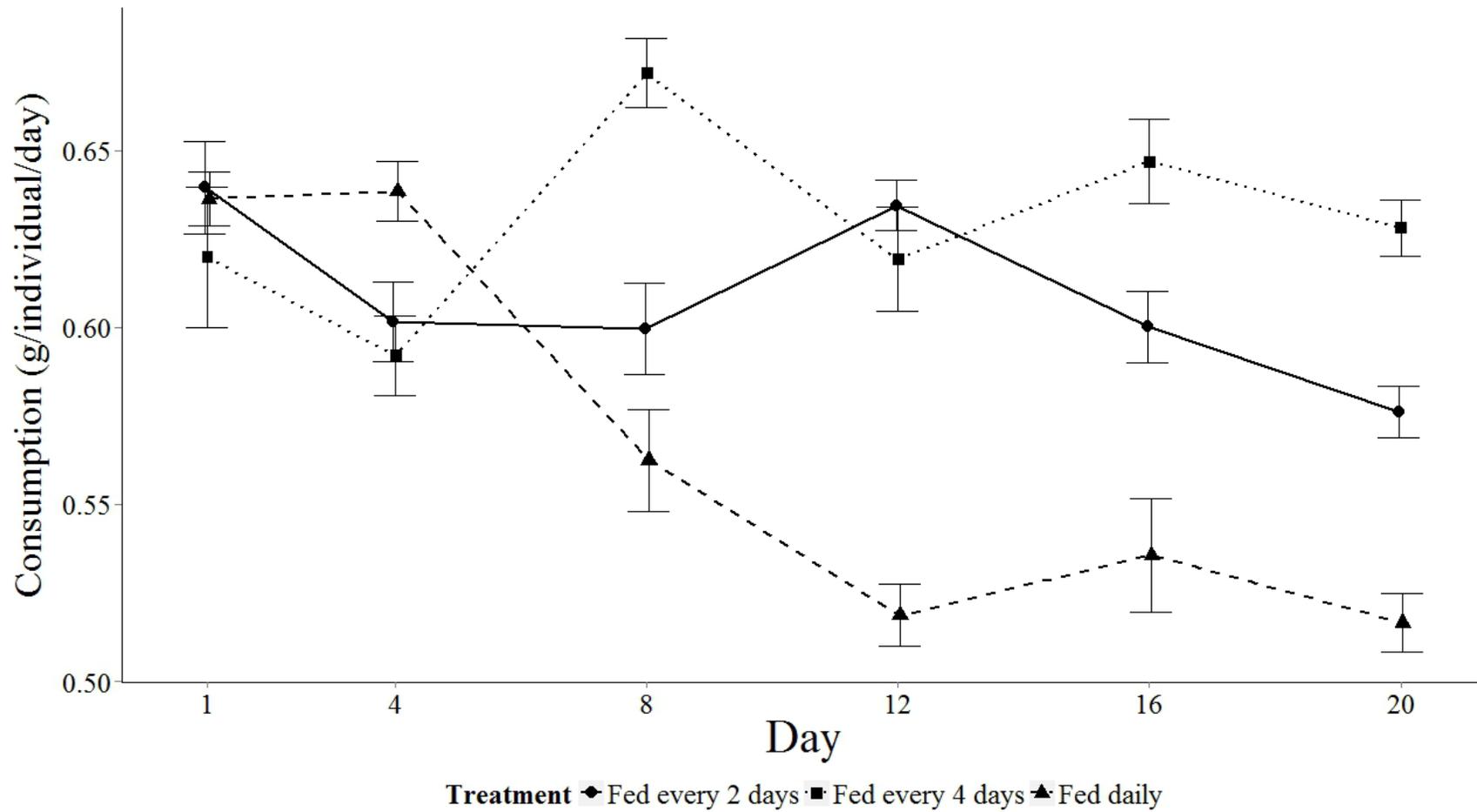


FIGURE 2.4. Mean food consumption rate (g/individual/day) of individuals fed ad libitum daily, every 2 days and every 4 days (Error bars=S.E.).

test of sphericity $p=0.005$). As a result, a correction (Huynh-Feldt) was employed to aid in interpreting within-subjects results (Table 2.2). Consumption significantly varied each day of the 20-day trial and between each treatment (Table 2.2). Consumption in individuals fed daily decreased with the progression of the 20-day study (Fig. 2.4) and significantly differed from all other treatments ($p<0.001$; Table 2.2). In comparison, consumption remained relatively constant for urchins fed every two days and even increased for urchins fed every four days (Fig. 2.4). Total consumption over 20 days was 9.24 g more for urchins fed every four days than those fed daily and 3.16 g more than those fed every two days.

Discussion

The ability to withstand periods of food shortage is a considerable asset for the survival of any animal (Wang et al. 2006). The present study showed that for *L. variegatus*, chemotaxis decreases with increased duration of food deprivation (Fig. 2.2). Many animals decrease physical activity during starvation to curtail energy loss, thereby increasing their tolerance to starvation (Van Dijk et al. 2002; Hervant et al. 2001). This has been documented in some fishes (Mendez and Wieser, 1993; Van Dijk et al. 2002) and amphibians (Hervant et al. 2001). However, other animals, such as rats (Koubi et al. 1991; Scalafani and Rendel 1978) and emperor penguins (Robin et al. 1998) increase physical activity at the later stages of starvation in what could be a final effort to find more food (Koubi et al. 1991; Pierre et al. 2001).

The act of foraging can be characterized as a matter of risks and gains (Tomba et al. 2001; Hay 2009; Weissburg 2000; Webster and Weissburg 2009). In contrast to starved and intermittently fed individuals, urchins that were fed daily travelled significantly faster (Fig. 2.2). The travel time for intermittently-fed individuals remained relatively constant throughout the study (Fig. 2.2) and could represent an attempt to balance nutritional needs and foraging risks.

Foraging for better food items (i.e., those with more available nutrients) while metabolic energy stores are available can promote starvation tolerance later (Wang et al. 2006). In the wild, increased movement could also improve the chance of encountering more palatable and nutritionally-replete food (vanDijk et al. 2002; Mendez and Wieser 1993).

Results from this study suggest that consumption for daily-fed *L. variegatus* is significantly lower than intermittently-fed individuals (Fig. 2.2). This decrease in feeding was not shown to be concomitant with a change in digestive capacity (Lawrence et al. 2003; Klinger and Lawrence 1985) and suggests that *L. variegatus*, in periods of food limitation, minimizes energy losses by limiting physical activity, but is prepared to consume food and assimilate nutrients at maximum rates when it becomes available. Intermittently-fed and starved *L. variegatus* appear to consume food, when available, beyond their capacity to utilize it for metabolic purposes (e.g., gonadal or somatic growth) (Lawrence et al. 2003; Klinger and Lawrence 1985). It is advantageous for animals in environments with limited and fluctuating food availability to consume large meals while food is still available followed by periods of decreased energy expenditure (McCue 2007; Wang et al. 2006).

Compensatory feeding response has been observed in many animals, especially herbivores, attempting to offset nutritionally-deficient food items or food limitation by increasing consumption when nutrient-dense food becomes available (Cruz-Rivera and Hay 2001; Robin et al. 1998; Jobling et al. 1993; Wu and Dong 2002). Similar mechanisms have been observed in other sea urchin species, such as *Paracentrotus lividus* (McCarron et al. 2009) and *Strongylocentrotus droebachiensis* (James and Siikavuopio 2012). Urchins are less selective during periods of food limitation (Fuji 1967; Deal 2003; Sakata 1989; McConnell 1982). Compensatory feeding response has been employed to optimize feeding regimes in other species

of aquacultured urchins (James and Siikavuopio 2012; McCarron et al. 2009; Xia et al. 2007) and could have similar implications for the husbandry of *L. variegatus*.

In summary, chemotaxis and duration of starvation appear to be inversely related for *L. variegatus*. In this study, *L. variegatus* decreased physical activity during prolonged food limitation, but maintained the ability to consume more food when it became available. Results from this study suggest that physiology and behavior are intimately related. The relationship between behavioral response and change in physiology during starvation need to be explored in future studies and could broaden understanding of sea urchin biology and ecology.

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CHAPTER III

THE ROLE OF AMINO ACIDS IN THE FORAGING AND FEEDING BEHAVIOR OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

Abstract

Foraging and feeding in sea urchins is largely governed by detection and response to chemical stimuli. Amino acids play a vital role in the olfactory and gustatory response of many marine organisms. This study evaluated the effects of several amino acids to the olfactory and gustatory response of the sea urchin *Lytechinus variegatus*. To evaluate olfactory response, sea urchins ($42.35 \pm 2.56\text{g}$; $n=168$) were exposed to one of eight concentrations (0.0000, 0.0001, 0.0010, 0.0100, 0.1000, 1.0000, 10.0000, 100.0000 mg/L) of six individual amino acids (L-alanine, L-arginine, DL-glutamate, glycine, L-leucine and L-tyrosine). The amount of time required to travel a fixed distance towards an amino acid source was used as a metric for olfactory response. To evaluate gustatory response, Type III phagostimulatory response (extension and repetitive opening and closing of the mouth) was measured by individually exposing sea urchins to similar dose and amino acid combinations as used to evaluate olfactory response. Responses were modeled using a four-parameter dose response curve. There was significant difference in the olfactory response between L-arginine and L-leucine ($p=0.009$), DL-glutamate and glycine ($p<0.001$), DL-glutamate and L-leucine ($p<0.001$), and L-alanine and L-leucine ($p=0.035$). The proportion of individuals that exhibited a Type III response was significantly lower for glycine than for L-tyrosine ($p=0.002$), L-leucine ($p=0.014$), DL-glutamate ($p=0.0085$) or L-alanine ($p=0.020$). This suggests that *L. variegatus* employ food-sourced amino acids to identify food sources and evaluate food palatability. Also, the concentration of chemical signals not just the compound in itself influence the type and intensity of the olfactory or gustatory response it evokes.

Introduction

Olfaction and gustation play a critical role in animal survival, allowing animals to detect and discriminate constituents of potential foods, as well as predators and toxins (Weissburg et al. 2002; Peacor and Werner 2001). Organisms, whether terrestrial or aquatic, are immersed in mixtures of chemical signals that convey various messages, from reproductive capacity (Sato and Goshima 2007) to food availability (Hay et al. 1986; Peacor and Werner 2001; Weissburg et al. 2002; Ferner and Weisburg 2005). Chemical signals can be subdivided into categories depending on the behavior they modulate. Repellents, attractants, and arrestants are compounds that stimulate the olfactory system and exert their influence from a distance (Lee and Myers 1996; Lindstedt 1971). Substances affecting taste and gustation function only when food is in contact with their receptors and are referred to as either incitants or deterrents, depending on their function (Lee and Myers 1996; Hay 2009). This study evaluated the manner in which select amino acids affect olfaction and gustation in the feeding behavior of the sea urchin *Lytechinus variegatus*.

Lytechinus variegatus is an echinoid found in a variety of shallow water habitats (Beddingfield and McClintock 2000). They are fast growing and omnivorous, consuming a variety of plant and animal matter (Beddingfield and McClintock 2000; Moore et al. 1963). Seagrasses such as *Thalassia testudinum*, *Cymodocea filiformis*, and *Halodule wrightii* are most commonly consumed (Moore et al. 1963; Beddingfield and McClintock 2000). At locations in which *L. variegatus* and mussels co-occur, the sea urchin will consume the whole mussel, shell, flesh and epibionts on the shell (Sklenar, 1994). Dietary selection in sea urchins is a balance of both availability and palatability of food sources (Hay et al. 1986; Dworjanyn et al. 2007) and they rely on their olfactory and gustatory systems to assess food availability, quality and palatability.

Amino acids play a vital role in initiating olfaction and gustation in crustaceans (Ache et al. 1976), mammals (Yarmolinsky et al. 2009; Nelson et al. 2008), echinoderms (Kidawa 2006; McClintock et al. 1984), and fish (Hara 2001; Laberge and Hara 2001). Specific olfactory and gustatory receptors for certain amino acids have been identified in mammals (Mayer 2005; Nelson et al. 2008; Chandrashekar et al. 2006), insects (Yarmolinsky et al. 2009), and fish (Hara 2006; Laberge and Hara 2001). The specificity and sensitivity of these amino acid receptors may be closely related and adapted to nutrient requirement, dietary choice and feeding history (Kidawa 2005, 2006; Hara 2006; McClintock et al. 1984; Smith and Travers 2008). For example, the catfish *Ictalurus punctatus* responds to most naturally-occurring amino acids, thus reflecting its broad range of dietary choices (Hara 2006). In contrast, salmonids respond to a relatively narrow spectrum of naturally occurring amino acids, due to its limited food preferences (Hara 2006).

Several studies have shown that sea urchins are capable of chemical perception (Klinger and Lawrence 1985; Vadas 1977; Hay et al. 1986; Dworjanyn et al. 2007); however, individual compounds have seldom been used as feeding attractants and feeding stimulants (Kidawa 2005; Sakata et al. 1989; Klinger and Lawrence 1984). Chemoreception studies in sea urchins commonly use either extracts from a common food item (e.g. Nagi and Kaneko 1975, Dworjanyn et al. 2007) or part of entire organisms (e.g. Hay et al. 1986) as feed attractants or stimulants. Also, majority of behavioral studies (e.g. Hay et al. 1986; Dworjanyn et al. 2007) on feeding attractants use ANOVA and linear regression techniques to describe responses to fixed amounts of a substance. However, many biological responses (e.g. enzyme kinetics, bacterial culture growth rate, receptor binding, etc.) are best characterized by a saturation curve (e.g. Michaelis-Menten equation, Hill equation), which is sigmoidal rather than straight-lined (Kepner

2010). Since olfaction and gustation rely on receptor binding (Chandrashekar et al. 2006; Smith and Travers 2008; Nelson 2002), it is possible that responses initiated by receptor-signal interaction exhibit saturation behavior. Information on the nature of effect of compounds on gustation and olfaction in sea urchins is lacking. Understanding the mechanisms that drive sea urchin feeding behavior could give a clearer picture of how animals perceive and respond to their environment. The objective of this study was to investigate the role and characterize the effects of several amino acids in foraging and feeding behaviors of the sea urchin, *Lytechinus variegatus*.

Materials and Methods

This study was conducted at the Texas A&M AgriLife Research—Shrimp Mariculture Project, Texas A&M System, Port Aransas, Texas, USA. Specimens of *L. variegatus* were supplied by the University of Alabama—Birmingham and were collected from Cape San Blas within the Port St. Joseph Peninsula State Park, FL, USA (30 °N, 85.5 °W). After receipt, sea urchins were maintained in a semi-recirculating system consisting of four 1.22-m diameter tanks equipped with one airstone, a central drain, an external standpipe, and an inflow water valve (Fig. 1.1). Seawater was pumped from the Corpus Christi Ship Channel and filtered through a 100- μ m filter prior to use. Water temperature, salinity, and dissolved oxygen in the tanks were monitored daily. Levels of nitrogenous metabolites (e.g., total ammonia nitrogen, nitrite) and pH were determined weekly. Water was introduced into maintenance tanks in order to create a circular current. Sea urchins were maintained on a 12h light: 12h dark photoperiod. Sea urchins were fed a standard reference diet (SRD) at ad libitum levels (Table 1.1). This diet is routinely used in all sea urchin research at the Shrimp Mariculture Project.

Olfaction Study

We measured the olfactory response of *L. variegatus* to individual amino acids introduced in graded concentrations (doses). The amount of time required for sea urchins to travel from the starting to the end point (Fig. 1.3; 1.4) served as the metric for quantification of olfactory response. Individual olfactory response was recorded as minutes post-placement. Those sea urchins unable to reach the end point were also noted. Each sea urchin was removed from the tank after it reached the end point or was unable reach the end point after three hr. After three hr, each tank was rinsed with distilled water then seawater to remove any residues (e.g. feed, spines, feces, etc.). A new trial was started after all the tanks had been rinsed. At the end of each trial day, tanks and all containers were rinsed with distilled water and dried overnight to minimize carryover.

This study was undertaken over six consecutive days (September 10-16, 2012). Individual amino acids (L-alanine, L-arginine, L-leucine, L-tyrosine, DL-glutamate, and glycine) were evaluated each day at eight doses (0.0000 [seawater], 0.0001 mg/L, 0.0010 mg/L, 0.0100 mg/L, 1.0000 mg/L, 10.0000 mg/L, and 100.0000 mg/L). Appropriate doses were achieved through serial dilution of 100 mg/L stock solutions. System seawater was used as diluent and as control treatment (0.0 mg/L) to distinguish response above that of ambient amino acid levels.

One sea urchin was assigned to each amino acid and dose combination (n=168) and used only once in the study. Each amino acid and dose combination had four replicates. On each trial day, each amino acid and dose combination being tested was randomly assigned to an experimental tank (Fig. 1.3; 1.4). Each experimental tank was black in color and measured 15.24 cm (W) × 45.72 cm (L) × 10.16 cm (D). One liter of the appropriate dose was introduced into the experimental tanks at a rate of approximately 5ml/min using 3.2-mm (inner diameter) tubes

equipped with pinch valves to regulate flow and minimize contamination (<http://www.carefusion.com/>). Release rate of the amino acids into each experimental tank was measured between each trial by catching all the liquid released into the experimental with a graduated cylinder for one minute. When necessary, release rate was adjusted using the pinch valves.

Olfactory response was modeled using a four-parameter dose response curve with the equation:

$$f(x, (b, c, d, e)) = c + \frac{d - c}{1 + \exp\{b(\log(x) - \log(e))\}}$$

where b is the slope, c is the lower limit, d is the upper limit and e is the 50% Effective Dose (ED50 hereafter). The four-parameter dose response curve was constructed using the *drc* package in R (Ritz and Streibig 2005; <http://cran.r-project.org>). The ratios between ED50 and slope estimates were calculated and a t-test was used for pair-wise comparison testing the null hypothesis that the ratio equals 1.

The resultant model estimates derived from the dose-response curve were used to describe the relative potency of the amino acid to elicit a response. The 50% Effective Dose or ED50 (mg/L) is an estimate of the concentration of amino acid required to produce 50% of that amino acid's maximum effect. The ED50 is halfway between the maximum and minimum response estimates (minutes). The slope estimate of the curve indicates how quickly or slowly maximal response is achieved after the first response is initiated. Sharply changing slopes suggest high potency. In such cases, maximum response is quickly achieved. In contrast, slopes that are not as steep are suggestive of

less potency where a greater change in dose is required to achieve maximal response. The dose-response curve also provides a minimum and maximum response estimate. These two parameters approximate the relative degree of response an amino acid elicits. For example, an amino with three times the maximum response estimate relative another amino acid is able to elicit a response three times greater than the other.

Phagostimulation Study

This study was used to measure the Type III phagostimulatory response of *L. variegatus* to different concentrations of individual amino acids. To test for phagostimulatory response, 10 mL of the appropriate dose of the amino acid being tested was introduced at a rate of about 2 mL/min around the peristome and mouth using a 10-mL syringe with an 18-gauge blunt-tip needle. Care was taken not to touch the animal with the implements being used. Qualitative assessment of a Type III phagostimulatory (i.e., full extension and repetitive opening and closing of the mouth) response (Klinger and Lawrence 1984) was observed up to three minutes after the last of the amino acid dose was expelled from the syringe.

This study was carried out over six consecutive days (September 10-16, 2012), testing one amino acid (e.g., L-L-alanine, L-arginine, L-leucine, L-tyrosine, DL-glutamate, and glycine) on each day with eight dosages (0.0000, 0.0001, 0.0010, 0.0100, 1.0000, 10.0000, and 100mg/L). From a pool of 200 sea urchins of similar weight (wet weight = 43.65 ± 3.24 g), 20 animals were randomly chosen each day for each dose of the amino acid being tested (20 animals/dose \times 8 doses/amino acid, n=160). Sea urchins were individually placed in containers (Hefty® 20 oz. styrofoam bowls) with seawater and allowed to acclimate for 30 min prior to testing. Seawater was not introduced into the containers through the duration of the study.

The proportion of animals displaying the Type III response was used to model gustatory response. Gustatory response was modeled using a 4-parameter dose response curve with the equation:

$$f(x, (b, c, d, e)) = c + \frac{d - c}{1 + \exp\{b(\log(x) - \log(e))\}}$$

where b is the slope, c is the lower limit, d is the upper limit and e is the 50% Effective Dose (ED50). The four-parameter dose response curve was constructed using the *drc* package in R (Ritz and Streibig 2005; <http://cran.r-project.org>).

Results

Olfactory study

Olfactory response of *L. variegatus* to graded concentrations of several individual amino acids was determined by measuring changes in travel time towards individual amino acid sources. The responses were described using a four parametric dose-response curve. The results of this study suggest that the amino acids tested required significantly different ED50 concentrations to elicit an olfactory response in *L. variegatus* (Table 3.1). There were significant differences in the ED50 estimates between L-arginine and L-leucine ($p=0.009$), DL-glutamate and glycine ($p<0.001$), DL-glutamate and L-leucine ($p<0.001$), and L-alanine and L-leucine ($p=0.035$; Table 3.1). Of all the amino acids tested, DL-glutamate was most potent ($ED50= 3.51 \times 10^{-4} \pm 2.53 \times 10^{-4}$ mg/L), requiring the smallest dose to achieve maximal response (Fig. 3.1; Table 3.1), whereas L-leucine required the highest dose ($ED50= 3.05 \times 10^{-2} \pm 2.24 \times 10^{-2}$ mg/L) to achieve maximal response (Fig. 3.1; Table 3.1).

There were apparent differences in curve slopes based on the shapes of the fitted curves (Fig. 3.1); however, no significant differences were detected between the slope estimates of each

curve using the statistical methods employed (Table 3.2). This lack of significant difference in slopes could be due to the high variability (increased S.E.) observed in the parameter estimates (Table 3.3). However, graphical representation (Fig. 3.1) of the data suggests that the slopes of glycine and L-tyrosine were not as steep as those of L-alanine, L-arginine, DL-glutamate and L-leucine. This suggests that relatively smaller changes in dose of these amino acids are required to achieve maximal response. Also, *L. variegatus* may be less sensitive to changes in glycine and L-tyrosine concentrations (Fig. 3.1; Table 3.2).

Phagostimulation Study

The number of urchins displaying Type III phagostimulatory response (i.e., full extension and repetitive opening and closing of the mouth) was determined after exposure to graded doses of individual amino acids. Analysis of results indicated no significant differences in the potency of the amino acids tested to initiate a phagostimulatory response in *L. variegatus* ($p > 0.050$; Fig. 3.2). The ED50 and slope estimates (Table 3.4) for each amino acids were not significantly different from each other ($p > 0.05$). The proportion of individuals exhibiting Type III response was significantly lower for glycine than for L-tyrosine ($p = 0.002$), L-leucine ($p = 0.014$), DL-glutamate ($p = 0.0085$) and L-alanine ($p = 0.020$). This suggests that although the potency of the amino acids tested was similar, the efficacy of glycine in eliciting Type III phagostimulatory response in *L. variegatus* is significantly lower compared to the other amino acids tested (Fig. 3.2).

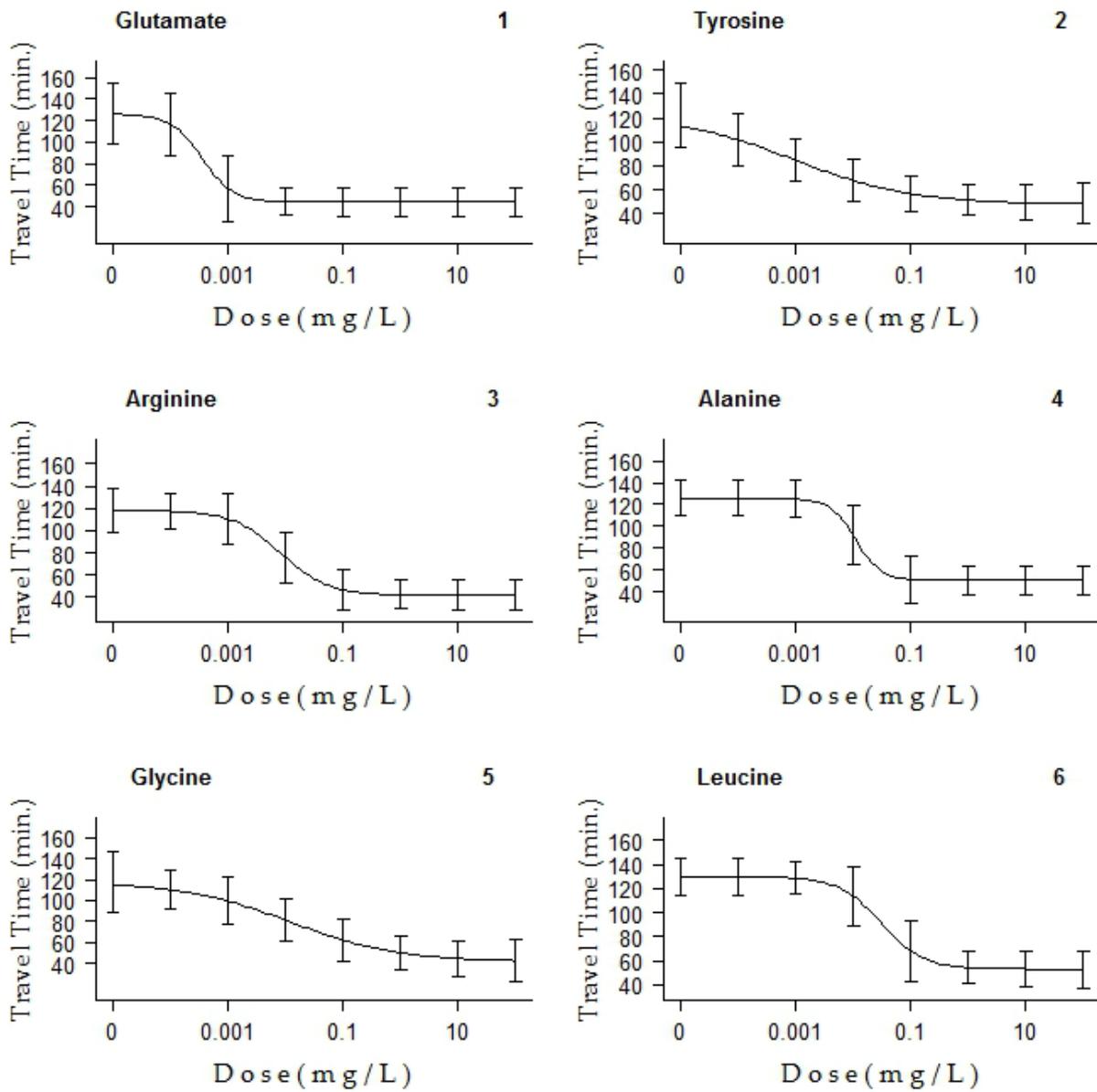


FIGURE 3.1. Fitted four-parameter dose response curves of *L. variegatus* to amino acids (\pm S.E.). Panel numbers represent order of attractiveness based on ED50 (see TABLE 3.3 for estimates).

TABLE 3.1. Pair-wise comparison of ED50 estimates for the olfactory response of *L. variegatus* to different amino acids.

	Estimate	S.E.	t-value	p-value
ala/arg	1.362	1.163	0.311	0.756
ala/glu	31.420	25.879	1.175	0.242
ala/gly	0.891	1.377	-0.079	0.937
ala/leu	0.361	0.302	-2.118	0.036
ala/tyr	11.458	18.947	0.552	0.582
arg/glu	23.075	24.096	0.916	0.361
arg/gly	0.654	1.095	-0.316	0.753
arg/leu	0.265	0.279	-2.630	0.009
arg/tyr	8.415	14.927	0.497	0.620
glu/gly	0.028	0.047	-20.658	0.000
glu/leu	0.011	0.012	-83.599	0.000
glu/tyr	0.365	0.642	-0.990	0.324
gly/leu	0.405	0.675	-0.882	0.379
gly/tyr	12.860	28.197	0.421	0.675
leu/tyr	31.734	56.005	0.549	0.584

TABLE 3.2. Pair-wise comparison of slope estimates for the olfactory response of *L. variegatus* to different amino acids.

	Estimate	S.E.	t-value	p-value
ala/arg	1.935	6.331	0.148	0.883
ala/glu	1.263	4.087	0.064	0.949
ala/gly	4.371	14.120	0.239	0.812
ala/leu	1.725	5.541	0.131	0.896
ala/tyr	4.806	15.396	0.247	0.805
arg/glu	0.653	0.731	-0.475	0.635
arg/gly	2.259	2.498	0.504	0.615
arg/leu	0.891	0.937	-0.116	0.908
arg/tyr	2.483	2.544	0.583	0.561
glu/gly	3.460	3.436	0.716	0.475
glu/leu	1.366	1.272	0.288	0.774
glu/tyr	3.805	3.429	0.818	0.415
gly/leu	0.395	0.361	-1.677	0.095
gly/tyr	1.100	0.972	0.102	0.919
leu/tyr	2.786	2.267	0.788	0.432

TABLE 3.3. Model parameter estimates from the dose response curve analysis for the olfactory response of *L. variegatus* to different amino acids.

	Slope		Lower Limit (minutes)		Upper Limit (minutes)		ED50 (mg/L)	
	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Alanine	2.05	6.45	50.38	6.94	125.54	8.35	1.10×10^{-02}	4.39×10^{-03}
Arginine	1.06	0.91	41.80	7.31	118.16	10.18	8.10×10^{-03}	6.12×10^{-03}
Glutamate	1.62	1.15	44.58	5.92	126.12	12.85	3.51×10^{-04}	2.53×10^{-04}
Glycine	0.47	0.32	41.43	11.72	117.49	13.28	1.24×10^{-02}	1.85×10^{-02}
Leucine	1.19	0.71	53.16	7.94	129.94	7.94	3.05×10^{-02}	2.24×10^{-02}
Tyrosine	0.43	0.23	47.89	9.21	122.43	12.89	9.62×10^{-04}	1.54×10^{-03}

TABLE 3.4. Model parameter estimates from the dose response curve analysis for Type III phagostimulatory response of *L. variegatus* to individual amino acids

	Slope		Lower Limit		Upper Limit		ED50 (mg/L)	
	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Alanine	-0.605	0.220	-0.016	0.083	0.909	0.065	4.20×10^{-03}	2.90×10^{-03}
Arginine	-0.530	0.205	-0.011	0.076	0.892	0.088	8.67×10^{-03}	8.08×10^{-03}
Glutamate	-0.531	0.207	0.019	0.094	0.962	0.070	5.46×10^{-03}	4.18×10^{-03}
Glycine	-0.728	0.290	-0.009	0.084	0.669	0.052	1.59×10^{-03}	1.32×10^{-03}
Leucine	-0.539	0.191	-0.007	0.074	0.888	0.085	1.48×10^{-02}	1.30×10^{-02}
Tyrosine	-0.952	0.449	-0.018	0.088	0.887	0.049	5.47×10^{-04}	2.68×10^{-04}

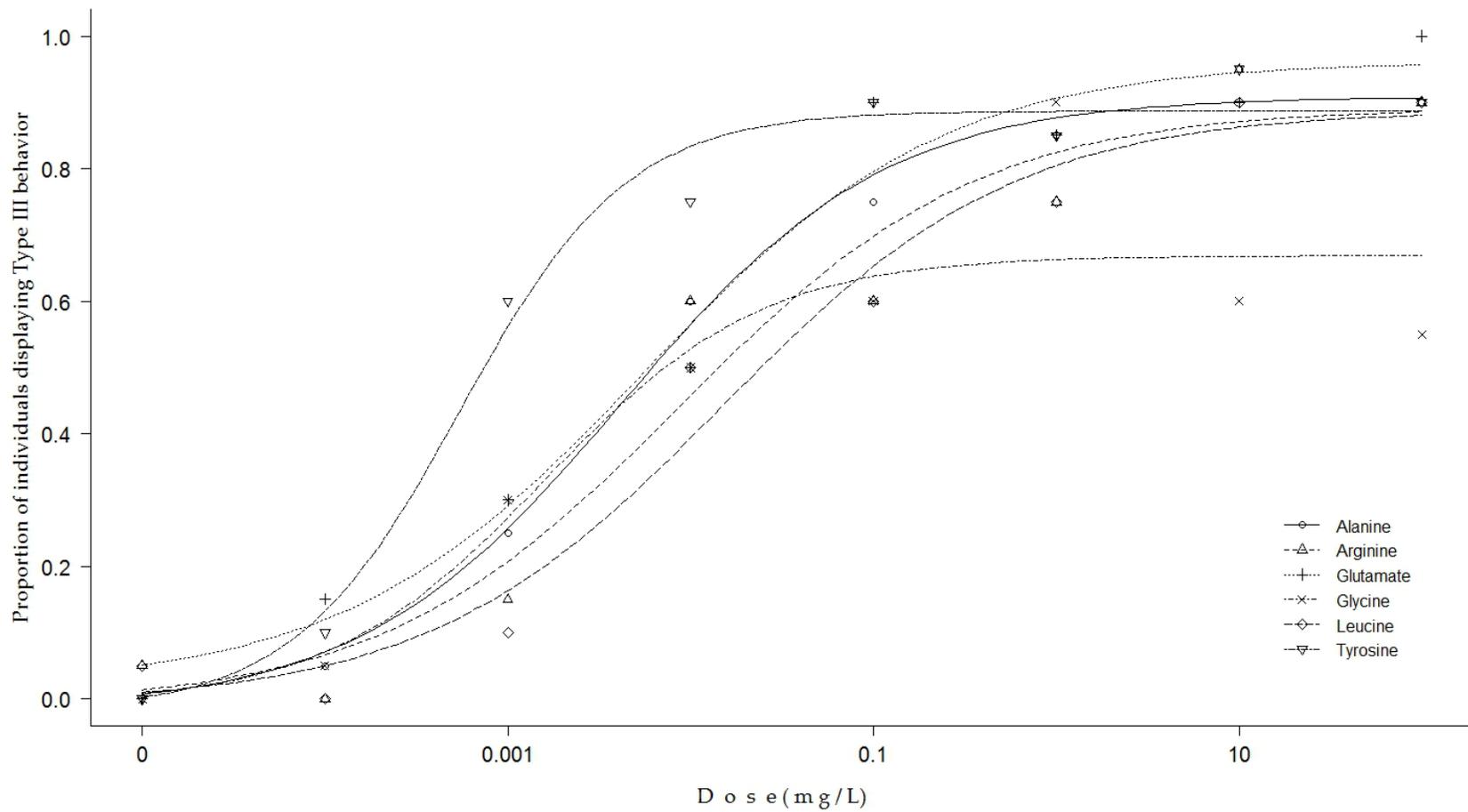


FIGURE 3.2. Fitted 4-parameter dose response curves for the phagostimulatory response of *Lytechinus variegatus* to several amino acids.

Discussion

Olfaction and gustation play a critical role in animal survival, allowing animals to detect and discriminate constituents of potential foods, as well as predators and toxins. Results of this study indicate a dose-dependent response of *L. variegatus* to amino acids (Fig. 3.1, 3.2). These results were similar to those of the sea star, *Odontaster validus*, when exposed to amino acids, either singly or in mixtures (Kidawa 2005, 2006). Specificity and sensitivity of detection thresholds for amino acids may be closely related and adapted to dietary choice and history (Kidawa 2005, 2006; Hara 2006; McClintock et al. 1984) as well as nutrient requirements (Smith and Travers 2008). For example, mammals are able to associate different tastes with nutrient groups in food: sweet for carbohydrates, salty for electrolytes, sour for acids, umami or savory for amino acids (particularly L-glutamate and L-aspartate in humans), and bitter for corrosive and potentially toxic substances (Chandrashekar et al. 2006; Smith and Travers 2008). Echinoids have also been observed to prefer palatable seaweeds (Dworjanyn et al. 2007; Hay et al. 1986) and are able to discriminate between more and less nutritious seaweeds (Hay et al. 1986). Amino acids could play an important role in this differentiation, providing a vital mechanism for *L. variegatus* to secure a diet that promotes homeostasis and avoids toxic substances.

In the current study, it was evident that certain amino acids elicited a stronger response than others. Olfactory response was most pronounced for DL-glutamate compared to other amino acids tested (Fig. 3.1). Similarly robust responses to glutamate have been observed in fish (Hara 2006), sea stars (Kidawa 2005; McClintock et al. 1984), mice (Nelson et al. 2002), and humans (Chandrashekar et al. 2006). Lobsters (Johnson et al. 1984), fish (Hara 2005), and mammals (Chandrashekar et al. 2006; Smith and Travers 2008; Nelson 2002) have dedicated receptors for glutamate (Mayer 2005).

Different organisms use different sets of odorants as cues and the dose of a single amino acid may not be as important as the ratio of amino acids in a mixture (Hay 2009). Amino acids seldom occur singly; rather, they are released from tissues in mixtures, and responses to single peptides are not as robust as those to blends (Rittschof and Cohen 2004; Hay 2009; Weissburg et al. 2002; Kidawa 2005). Odor and taste representations are generated by a convergence of sensory inputs from several receptors (Su et al. 2009; Chandrashekar et al. 2006; Hara 2005). Animals have evolved several receptor suites with specificities and sensitivities that best meet their physiological and environmental demands (Smith and Travers 2008; Su et al. 2009). Receptor repertoire in invertebrates may not be as complex as those in vertebrates (Yarmolinsky et al. 2009), but may be analogous. Despite the differences in receptor suites between phyla, nutritionally important compounds are categorized based on similar sensory inputs: bitter for potentially toxic compounds, sweet for carbohydrates and salty for electrolytes (Yarmolinsky 2009; Chandrashekar et al. 2006; Su et al. 2009).

Olfactory and gustatory response, although different for each species, is innate and reflexive (Su et al. 2009; Yarmolinsky 2009; Weissburg et al. 2002; Smith and Travers 2008; Hay 2009). In this study, the potency of each amino acid needed to elicit a response was different but the triggered reaction was the same (chemotaxis or phagostimulation). Analogous responses have been noted in fish (Hara 2001; 2005), asteroids (McClintock et al. 1984; Kidawa 2005), crustaceans (Moore and Grills 1999), mammals (Su 2009) and other echinoids (Hay et al. 1986; Dworjanyn et al. 2007). However, these innate behaviors can be modified through learning (Yarmolinsky et al. 2009). For example, sea urchins appear to consume more nutritious algae over chemically-defended ones (Hay et al. 1986; Dworjanyn et al. 2007). This plasticity in

feeding behavior allows organisms to adapt to ever-changing physiological and environmental conditions.

Most biological responses (e.g. enzyme kinetics, bacterial culture growth rate, receptor binding, etc.) are best described by a saturation curve (e.g. Michaelis-Menten equation, Hill equation) rather than a straight line (Kepner 2010). Since chemical detection is receptor driven (Chandrashekar et al. 2006; Smith and Travers 2008; Nelson 2002), it is probable that the behavior initiated from the reception of these signals follow a saturation curve. Although, there is high variability in the parameter estimates (Table 3.3, 3.4), results from this study suggest that behavioral response in *L. variegatus* to graded concentrations of amino acids follows a sigmoidal curve. Previous behavioral studies (e.g. Hay et al. 1986; Dworjanyn et al. 2007) on feeding attractants employed ANOVA and linear regression to differentiate palatability and attraction between fixed amounts or concentrations of a substance. In contrast, by using several doses of a substance, the effective dose, minimum and maximum response can be estimated. This is an improvement over the generally qualitative measurements utilized in previous studies. These estimates can be used to identify specific concentrations and substances needed to elicit a behavioral response, analogous to the determination of the nutrient level requirement of an animal or the specific therapeutic dose of a drug. The estimates can also be useful when comparing results between studies, test systems, and organisms. Similar standardized measures are already used in pharmacology and toxicology (e.g. Pelham et al. 2014, Kniss et al. 2011) and nutrition research (Shearer 2000). Although some studies (e.g. Kidawa 2005, 2006, Klinger and Lawrence 1984, 1985) have used comparable techniques, dose response curve analysis is still not widely utilized in ecological studies. However, for improved utility in the future, methodological

refinements in testing and analysis are still necessary to minimize variability and improve the accuracy of model parameter estimates.

Amino acids are effective olfactory and gustatory cues to the sea urchin, *L. variegatus*. All amino acids tested elicited, with varying intensities, positive olfactory and gustatory responses. This supports the hypothesis that *L. variegatus* employs amino acids to cue on food sources and to assess food palatability. However, additional research needs to be undertaken with *L. variegatus* to evaluate response to amino acid mixtures. The mechanisms by which detection and interpretation of these signals affect behavior still need to be explored. Further understanding of the effects and mode-of-action of feeding attractants is also necessary.

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CONCLUSIONS

The ecological niche an organism fills is greatly influenced by its feeding behavior. For *Lytechinus variegatus* this behavior is largely guided by the detection and response to chemical stimulus. The purpose of this dissertation is to characterize the olfactory and gustatory response of *L. variegatus* to specific chemical stimuli. A series of experiments were performed to evaluate the effects of flow rate and hunger on the chemical response of *L. variegatus*. Olfactory and gustatory response was evaluated by exposing *L. variegatus* to one of eight concentrations of six individual amino acids (L-alanine, L-arginine, DL-glutamate, glycine, L-leucine and L-tyrosine). The results of these studies indicate that 1) *L. variegatus* is positively rheotactic and, under the conditions of the study, increases consumption rate with increased flow rate; 2) *L. variegatus* decreases physical activity during periods of prolonged food limitation, but consume larger amounts of food when it becomes available; and 3) *L. variegatus* employ specific amino acids differently to cue on food sources. These findings could help expose components of a scantily studied mechanism that drives sea urchin navigation, foraging, distribution, food choice and interactions. These findings could also impact sea urchin aquaculture, affecting both feed formulation and sea urchin husbandry techniques.

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