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Effects of Fluctuating Salinity, Nutritional State, and Temperature on Leptasterias Spp. From Little Port Walter, Alaska.

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EFFECTS OF FLUCTUATING SALINITY, NUTRITIONAL STATE, AND TEMPERATURE ON LEPTASTERIAS SPP. FROM LITTLE PORT WALTER, ALASKA

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in
The Department of Biological Sciences

by
Jeffrey William Tamplin
A.B., Augustana College, 1986
M.S., Louisiana State University, 1988
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LIST OF ABBREVIATIONS AND SYMBOLS

\( \text{cm} = \) centimeter
\( d = \) day
\( ^\circ C = \) degrees celcius
\( \text{DNA} = \) deoxyribonucleic acid
\( g = \) gram
\( \geq = \) greater than
\( \text{hr} = \) hour
\( < = \) less than
\( \text{LC}_{50} = \) lethal concentration; concentration (or temperature) at which 50\% of individuals sampled die
\( \mu = \) micro
\( \mu lO_2\cdot hr^{-1} = \) microliters of oxygen consumed per hour
\( \mu lO_2\cdot hr^{-1}\cdot g^{-1} = \) microliters of oxygen consumed per hour per gram of tissue
\( \text{min} = \) minute
\( \text{ml} = \) milliliter
\( \text{mm} = \) millimeter
\( \text{mOsm}\cdot \text{kg}^{-1} = \) milliosmoles per kilogram
\( n = \) number
\( O_2 = \) oxygen molecule
\( Q_{10} = \) temperature quotient
\( ooS = \) salinity, measured as parts per thousand
\( \% = \) parts per hundred (percent)
\( p = \) probability
\( \text{PCR} = \) polymerase chain reaction
\( \text{PCR-RFLP} = \) polymerase chain reaction-restriction fragment length polymorphism
$\pm$ = plus or minus
$r^2$ = correlation coefficient
spp = species
SD = standard deviation
SE = standard error
$T_c$ = critical temperature; temperature outside the capacity adaptation of a species
$T_o$ = optimal temperature; preferred temperature of a species
ABSTRACT

The effects of fluctuating salinity, nutritional state, and temperature on activity, oxygen consumption, feeding, and growth rates were analyzed on seasonally collected Leptasterias spp. (Echinodermata: Asteroidea) from Little Port Walter, AK. Leptasterias aspera and L. epichlora were collected on June 10, 1994, divided into size groups, and perivisceral fluid osmolality was measured every three hours during a 12 h 30-10-30 o/o os fluctuating cycle. The perivisceral fluid osmolality of larger individuals fluctuated less than that of smaller sea stars. The perivisceral fluid osmolality of small and large Leptasterias spp. closely tracks the ambient water osmolality during tidal cycles.

Leptasterias spp. were collected on August 31, 1994 and separated into three nutritional treatment groups and analyzed over 31 days. Starved individuals had lower oxygen consumption rates than fed individuals. Feeding and starvation data suggest that Leptasterias spp. from Little Port Walter can survive beyond 31 days with a negative energy budget.

For studies of temperature acclimation, Leptasterias spp. were collected at the annual temperature minimum (1°C; 19 November 1995, 27 February 1998) and the annual temperature maximum (12°C; 1 September 1996, 2 July 1997). Individuals from seasonal collections were step-wise acclimated (2°C every two days) or acutely exposed to 5°C, 7.5°C, 10°C, 12.5°C, 15°C, 17.5°C, 20°C, or 22.5°C. The 28-day LC50 of winter acclimatized, acutely exposed animals were 8 and 10°C lower than summer acclimatized, step-wise acclimated and summer acclimatized, acutely exposed individuals, respectively.

Acute exposure and step-wise acclimation to experimental temperatures above the normal environmental maximum, 12.2°C, resulted in suppressed feeding and elevated oxygen consumption rates. Leptasterias spp. exist near the upper limit of capacity adaptation when environmental temperatures reach the annual summer
maximum and undergo seasonal acclimatization to water temperature. *Leptasterias* spp. are adapted to withstand sublethal temperature shifts, which occur both seasonally and diurnally in the intertidal zone.

Activity only partially correlated with environmental changes (temperature exposure, fluctuating salinity, and nutritional state of the animal). Reduced salinity negatively affected activity in *Leptasterias* spp. Activity coefficients varied with starved and fed feeding regimes, but were not significantly different between feeding treatments or time. Activity coefficients of *Leptasterias* spp. did not vary significantly with temperature.
INTRODUCTION

The rocky intertidal zone represents a complex and variable environment which exposes inhabitants to large variation in temperature, salinity, dissolved oxygen, wave action, humidity, solar radiation, and food availability. The severity of these environmental gradients strongly influences biochemical and physiological systems of intertidal organisms; adaptation to stressful levels of environmental factors is critical for survival in the intertidal zone (Newell 1979, Shirley and Stickle 1982a, Stickler and Bayne 1987, Denny 1988). Competition for space, food, and other resources are also important aspects of the rocky intertidal zone (Connell 1961a, b, Dayton 1971). The interaction of physical environmental factor gradients and biological relationships among intertidal species produces a complex network which determines the structure of intertidal communities. Because of the relative accessibility of coastal intertidal areas, as well as the pronounced ecological interactions between and within intertidal species, the dynamics between physical and biological factors characteristic of the rocky intertidal zone of the North American Pacific coast have received much attention (Dayton 1971, 1975, Hofmann and Somero 1995, Huey 1991, Lubchenco and Menge 1978, Menge 1972a, b, 1974, 1978, Menge and Menge 1974, Paine 1966, 1969, 1974, 1976, 1977, and Ricketts et al. 1985, Shirley and Stickler 1982a, b).

The effects of environmental factors on the physiology of marine invertebrates have been documented for a variety of mollusks, annelids, and crustaceans (Bayne 1975, Guerin and Stickler 1992, Johns 1981a, b, 1982, Schottler et al. 1983, Sommer et al. 1997, Stickler and Bayne 1982, Toulmond and Tchernigovtzeff 1984) and several species of echinoderms (Drouin et al. 1985, Greenwood and Bennet 1981, Lawrence 1975, Roller and Stickler 1993, Sabourin and Stickler 1981, Shirley and Stickler 1982a, b, Stickle and Diehl 1987). Much of this work has centered on temperature and salinity fluctuations and their effects on oxygen consumption, feeding rates, and other
physiological performance indices (scope for growth, absorbance efficiency, righting responses). Although most echinoderm species are considered to be strictly stenohaline, members of the phylum inhabit salinity extremes ranging from 5‰oS (*Amphipholis squamata, Ophiopholis aculeata, Strongylocentrotus droebachiensis*) to 60‰oS (*Astropecten* and *Asterina*) (Drouin et al. 1985, Stickle and Diehl 1987), thus suggesting that some adaptation and/or alteration of tolerance levels may exist within certain species of echinoderms. Because echinoderms possess rather limited physiological systems (lack of an excretory organ, poor ability to osmo- and ion-regulate), low salinity (< 30‰oS) often produces significant adverse morphological, physiological, reproductive, and developmental changes (Kinne 1971, Binyon 1972, Roller and Stickle 1985, 1989, and 1993). *Leptasterias hexactis* from southeastern Alaska tolerated 12.9‰oS for 21 days (Shirley and Stickle 1982a, b) and *Strongylocentrotus droebachiensis* and *Eupentacta quinquesemita* from southeastern Alaska tolerated 12 - 13‰oS for 28 days (Sabourin and Stickle 1981).

Coastal areas along southeastern Alaska are often fiord-like, and the combination of steep rocky physiography, spring snow and summer glacial melts, and high precipitation levels during late summer and early fall, promote the development of freshwater lenses. These lenses overlie full-strength seawater and produce intertidal zones which may be diurnally exposed to pockets and layers of reduced salinity. Stickle and Denoux (1976) concluded that echinoderms from southeastern Alaska may represent different physiological races of species than populations which are never exposed to reduced salinity. Stickle and Diehl (1987) report population differences in the salinity tolerance of *Eupentacta quinquesemita, Leptasterias hexactis, and Strongylocentrotus droebachiensis*. *Leptasterias hexactis* from Friday Harbor, Washington demonstrated a 28-day LC₅₀ of 16‰oS, while *L. hexactis* from a low and variable salinity environment

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near Juneau, Alaska displayed a 28-day LC50 of 13\%oS. *Strongylocentrotus droebachiensis* from both Friday Harbor and Juneau generated 28-day LC50 values of 12 - 13\%oS, but Lange (1964) reported a 28-day LC50 of 22\%oS from a population near Droebak, Norway. *Eupentacta quinquesemita* from Friday Harbor display limited tolerance to low salinity (28-day LC50 of 26\%oS), however a population from Juneau produced a 28-day LC50 values of 12 - 13\%oS. Similarly, Gezelius (1963) identified two European physiological races of the echinoid *Psammechinus miliaris* with contrasting responses to reduced salinity. These populations demonstrated the ability to cross-acclimate to altered salinity regimes in a period of 50 days. Data from Binyon (1961) supports similar inferences in the asteroid *Asterias rubens* from the Baltic and North Seas.

The evolutionary and metabolic importance of environmental stressors to intertidal invertebrates have recently received increased interest. Effects of temperature, particularly heat stress, have been a focal point of this research (Hoffman and Parsons 1991, Hofmann and Somero 1995, Huey 1991, Lenski and Bennett 1993). Thermal stress impacts physiological systems on both the molecular and cellular levels, but factors contributing to the metabolic cost of heat stress have not been completely evaluated (Hawkins 1991). Temperature is particularly important because shifts in temperature change the kinetic energies of the molecular systems on which physiological mechanisms depend (Klinger *et al.* 1986) and thermal damage is a significant source of protein denaturation (Hofmann and Somero 1995). However, little data exist as to whether thermal denaturation of proteins occurs in invertebrates during sublethal heat stress, i.e., under conditions which fall within the capacity adaptation of the organism.

Thermal stress may play an important role in protein denaturation and metabolic failure due to several factors: 1) thermolability of proteins (the weak bonding
arrangements and interactions which stabilize higher order protein structure are strongly affected by temperature shifts (Somero 1995); 2) the thermostability of proteins can be positively correlated with acclimatization temperatures (Jaenicke 1991, Somero 1995); and 3) the energy required to replace damaged proteins ranges from 18 to 26% of total metabolic heat loss in ectotherms (Hawkins 1985). Changes in the protonation of macro- and micromolecules, and hence the acid-base status of biochemical systems, are also important effects of temperature on physiological systems (Sommer et al. 1997). For example, Reeves (1972) demonstrated in bullfrogs that pH in various body compartments changes with temperature at -0.017 pH units per °C (ΔpH/°C), so that the protonation state of enzyme histidine imidazole residues is maintained at a constant level. The constant degree of protonation maintains protein function (Sommer et al. 1997). Because values of ΔpH/°C differ between tissues and blood plasma, Butler and Day (1993) concluded that extra- and intracellular pH may be independently regulated.

In temperate habitats, intertidal echinoderms are often exposed to large and rapid ambient temperature changes dictated by tidal cycles. Few data are available on the changes of body temperature in intertidal invertebrates during seasonal and tidal cycles (Hofmann and Somero 1995). Intertidal echinoderms may be seasonally exposed to air temperatures extremes between 0°C and 35°C for several hours a day (Farmanfarmaian 1966), followed by rapid inundation in water of vastly different temperature. Investigations on thermal tolerance and acclimation in echinoderms indicates exponential changes in oxygen consumption rates within temperature ranges typical for the species (Farmanfarmaian and Giese 1963, Choe 1962, Fuhrman and Fuhrman 1959). Apparently, metabolic adjustment to thermal stress is a primary evolutionary factor which facilitates survival in the intertidal zone.

Temperature significantly affects the feeding rate and righting response of several asteroid species (Kleitman 1941, Tamplin and Stickle 1998). The influence of
temperature on several metabolic processes (feeding rate, digestion and absorption rates, oxygen consumption rates, energy partitioning) has been reported for a number of echinoderm species. Although the effects of temperature on survival, developmental processes, and nutrition are fairly well documented for the echinoids *Strongylocentrotus* and *Lytechinus* (Farmanfarmaian 1966, Farmanfarmaian and Giese 1963, Lawrence 1975, Percy 1973, Roller and Stickle 1993) a paucity of data regarding temperature and other environmental factor gradient effects exists for asteroid echinoderms (Stickle 1985). Lawrence (1985) and Shirley and Stickle (1982a, b) provide the only comprehensive bioenergetic data involving asteroid responses to environmental gradients. The effects of temperature on asteroid respiratory physiology are poorly known and data are limited to oxygen consumption studies by Koller (1930) and Meyer (1935).

*Leptasterias* is a circumboreal and circumpolar genus of generally small, five- or six-rayed intertidal or subtidal sea stars. Due to the broad distribution and polymorphic nature of the genus, systematics of the group have been controversial for decades and a variable number of subgenera, species, and forms have been described (Foltz 1998). The wide geographic distribution and the variety of habitats occupied by the genus, combined with the limited dispersal capability associated with brooding reproductive behavior, has brought several biological factors into play (local and regional adaptation, limited advanced generation hybridization) which serve to complicate population structure and systematic analysis of the genus.

Fisher (1930) presented the most comprehensive taxonomic revision of the genus from the North Pacific and D’yakonov (1950) summarized Asian species known from the coast of the former Soviet Union and northern Japan. Fisher (1930) recognized eight intertidal *Leptasterias* species, most containing several named subspecies and forms. Chia (1966) synonymized several of Fisher’s (1930) North Pacific...
Pacific intertidal species into the extremely polymorphic *L. hexactis*. Current systematic publications (see Kwast et al. 1990, and Foltz et al. 1996a, 1996b) recognize four intertidal species from the temperate and subarctic North American Pacific coast: *L. aequalis* (Stimpson 1862b), *L. epichlora* (Brandt 1835), *L. hexactis* (Stimpson 1862a), and *L. pusilla* (Fisher 1930). Additionally, Foltz and Stickle (1994) assigned previously recognized Alaskan and British Columbian *L. hexactis* to *L. aspera* [Fisher’s (1930) *L. hexactis* forma *aspera*].

Kwast et al. (1990) analyzed 14 allozyme loci and identified two “pure” species (*L. hexactis* and *L. epichlora*) and a third presumptive species (*L. aequalis*), inferred to be a *hexactis* x *epichlora* hybrid, from five locations in southern Alaska and four sites in the Puget Sound, Washington. Subsequently, Foltz and Stickle (1994) segregated *L. aequalis* as a species distinct from *L. epichlora* x *hexactis* hybrids. Hrincevich and Foltz (1996) and Foltz et al. (1996a, 1996b) analyzed mitochondrial DNA of intertidal *Leptasterias* spp. using PCR-RFLP (restriction fragment length polymorphisms) and direct sequencing of the amplified PCR product to identify 11 mitochondrial DNA haplotypes (A-K) which act as biological species. Foltz (1998) summarized earlier studies and compared mtDNA haplotype designations with the earlier allozyme-based species designations. *L. aequalis* is composed of 96% haplotype A, but includes some B and C haplotypes. *L. aspera* is composed of haplotypes E, F, and H; *L. epichlora* was 98% composed of C and G but also encompassed A, B, F, and I; *L. hexactis* consisted mostly of haplotype A, with 18% B, and 6% D. Phylogenetically, haplotypes E, F, and H (*L. aspera*) form a clade, as well as haplotypes C and G (*L. epichlora*). The remaining haplotypes which also form a clade (A, B, D, and K) have been previously identified as either *L. aequalis* or *L. hexactis* (Foltz 1998).

*Leptasterias* ranges along the Pacific coast of North America from the Aleutian Islands, Alaska, to central California. The ranges of *Leptasterias hexactis*, *L. epichlora*,

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and *L. aequalis* overlap on Vancouver Island, British Columbia. *Leptasterias epichlora* ranges to the north, *L. aequalis* to the south, and *L. hexactis* in both directions. In addition to these broadly distributed species, *L. pusilla* is known from the Monterey Bay region of southern California. Extensive collections have been made throughout the range, and in 50 locations, a maximum of three species were in sympathy at any one location (Foltz and Stickle 1994, Foltz 1998). Hybridization is rare in the sympatric areas (only 3.2% of 2,540 total animals surveyed) and occurred at only 9 of the 50 locations. The distributional patterns of the *Leptasterias hexactis* species complex suggest that competition for resources and tolerance of environmental gradients may play a role in the interactions of *Leptasterias* species.

*Leptasterias* spp. (Fig 1.1), an intertidal species of six-rayed sea star, is an ecologically important predator common in the Aleutian (Northern) zoogeographical province. Individuals are extremely abundant along the rocky shores of the North American Pacific coast. This species is found in either brackish water or full strength seawater on sheltered rocky beaches (Stickle and Foltz 1994). *Leptasterias* spp. broods its eggs and shows high interpopulation genetic variability relative to several Aleutian province broadcast fertilizing asteroids (Stickle et al., 1992).

In many areas along the Pacific coast of North America, *Leptasterias* spp. and several other asteroids (such as *Evasterias troschelii* and *Pisaster ochraceus*) strongly influence the community and population structure of intertidal areas. *Leptasterias* commonly feeds on barnacles, limpets, the bivalve *Mytilus trossulus*, and a variety of other prey determined by habitat type and prey availability factors (Mauzey et al. 1968, Menge 1972a, b). In rocky and cobble intertidal habitats, *Leptasterias* predation is a primary factor influencing the abundance, age structure, and zonation of intertidal *Mytilus*. Intraspecific competition for food produces complex patterns of spatial,
Figure 1.1  *Leptasterias* spp. from Little Port Walter, Baranof Island, Alaska.
temporal, and dietary overlap between *Leptasterias* individuals in the intertidal zone (Menge 1972b).

Sea stars analyzed in this study were collected from Little Port Walter estuary (Fig. 1.2). The estuary is located near the southern tip of Baranof Island, approximately 64 kilometers southeast of Sitka, Alaska. The coastline of the island is typical of southeastern Alaska and is periodically indented by long, narrow, deep embayments from which mountains rise sharply to heights of a thousand meters or more. Most of the coast, including that along Little Port Walter, consists of a rocky cobble substrate, large boulders, and a pronounced intertidal zone, usually rich in *Mytilus trossulus*, *Fucus* spp., *Pisaster ochraceus* or *Evasterias troschelli* and a variety of chitons, limpets, barnacles, and seaweed. Little Port Walter demonstrates a number of features common to the Pacific coast intertidal zone: fluctuations of environmental factor gradients including salinity, temperature, dissolved oxygen levels, and precipitation. A single tributary stream, Sashin Creek, enters Little Port Walter from the southwest. At the seaward end, the estuary connects with Chatham Straight and Big Port Walter Bay. A well-defined peninsula, The Neck, juts into the estuary from the eastern shore and divides the estuary into two bays of approximately equal area, Inner Bay and Outer Bay. A small channel, The Narrows, connects the two Bays and allows water exchange between them (Powers 1962). Inner Bay displays structural characteristics of a fiord. Outer Bay connects directly to Chatham Straight through a channel approximately 36 meters deep. Outer Bay is sheltered from Chatham Strait by three small rocky islands (Inner Island, Middle Island, and Outer Island). *Leptasterias* were collected along the western shore of Outer Bay, in an area partially sheltered by these islands (Fig. 1.3). The maximum range of spring tides is 4.6 m; the maximum range of neap tides is less than 50% of the spring tides.
Figure 1.2  Map of the Pacific coast from Washington to Alaska. Inset map indicates collection site (Little Port Walter estuary, Baranof Island) along the southeast coast of Alaska.
Figure 1.3 Little Port Walter estuary, Baranof Island, southeastern Alaska.
In addition to a high tidal amplitude, Little Port Walter displays vertical salinity stratification (Powers 1962). Little Port Walter receives heavy precipitation (average = 221 inches/year) particularly during the late summer and fall months (July - October), producing a distinct freshwater lens which overlies seawater. Surface salinity during the summer ranges from 2%/ooS at the mouth of Sashin Creek to 29%/ooS at the mouth of Outer Bay. Strong vertical stratification exists in the estuary, particularly in Inner Bay. Water of less than 30%/ooS is generally found in the upper 2 m of Inner Bay and not below 5 m. Vertical stratification in Outer Bay is less pronounced, but low salinity water usually occurs in the upper 2 m.

Water surface temperature varies seasonally and ranges in summer from highs (>12.5°C) at the mouth of Outer Bay to lows (>10.5°C) at the mouth of Inner Bay. Powers (1962) determined that relatively warm water (>12.5°C) protrudes sharply into the southeastern quarter of Outer Bay from Chatham Straight. Slightly cooler water (>11°C) extends through The Narrows into the outer end of Inner Bay, then gradually warms toward the Sashin Creek outlet. Water temperatures drop with increased depth in both bays; the thermocline is steeper in Inner Bay (11°C - 8°C) than in Outer Bay (12°C - 10°C) from surface water to a depth of 25 meters.

Water temperatures at Inner Bay are nearly isothermal, with some slight stratification forming during the latter part of April and early June (Powers 1962). Until early June, surface water was colder than water slightly below the surface. The highest temperatures observed, 6°C to 7°C, were at a depth of 2 meters. Rapid warming occurred in mid-June and July. The highest water temperature recorded by Powers (1962), 12.2°C, occurred on July 11. This warming trend coincided with increased air temperatures, which peaked at 21.1°C on July 10. Alternating trends of slightly warmer and cooler water were linked with fluctuating air temperatures during the rest of the summer.
*Leptasterias epichlora* and *L. aspera* are common in the rocky intertidal zone of Little Port Walter. *Leptasterias* individuals are more common in Outer Bay and along the shores of the three islands than in Inner Bay. In this area, *Leptasterias* are exposed to fluctuating salinity, particularly in the late summer-early fall (July-September). At Little Port Walter, *Leptasterias* preys mainly on the abundant Blue Mussel, *Mytilus trossulus*, and competes for food and space resources with the asteroids *Evasterias troschelii* and *Pisaster ochraceus*.

Intertidal *Leptasterias* from Little Port Walter are subjected to a variety of environmental factor gradients, particularly those related to temperature, salinity, and dissolved oxygen. Little Port Walter displays fluctuating levels of water temperature, air temperature, salinity, and dissolved oxygen, based not only on pronounced seasonal patterns, but also on daily and monthly cycles determined by specific interactions of several of environmental factors. Intertidal *Leptasterias* at Little Port Walter are exposed to water temperatures as low as 0°C - 1°C in the winter months followed by gradual warming, culminating in exposure to water temperatures of 12°C or higher during the summer months. Air temperatures range from well below freezing during the winter to temperatures just over 20°C in the summer. Intertidal animals at Little Port Walter are exposed to air temperatures during low tides for periods of several hours followed by immersion in water which is often at vastly different temperatures than the prevailing air temperature. Little Port Walter was chosen as the collecting site for this study because its intertidal inhabitants are exposed to pronounced and documented (Powers 1962) diurnal and seasonal changes in environmental factor gradients.
SIZE EFFECTS ON THE PERIVISCERAL FLUID OSMOLALITY OF TWO SPECIES OF SIX-RAYED SEA STARS (Leptasterias spp.) EXPOSED TO FLUCTUATING SALINITY

INTRODUCTION

Salinity fluctuations have been shown to have a pronounced effect on the perivisceral fluid osmolality of many marine invertebrates (Stickle and Ahokas 1974, Stickle and Denoux 1976, Hand and Stickle 1977, Findley et al. 1978, Findley and Stickle 1978, Hildreth and Stickle 1980, Shirley and Stickle 1982a, b, and Sabourin and Stickle 1981). In nature, salinity fluctuations are often related to tides, glacial melt patterns, prevailing currents, precipitation levels, and flow rates in estuarine habitats. Often, estuarine areas are vertically stratified in temperature and salinity; this increases the probability of exposure to changing ambient salinity (Stickle and Denoux 1976). Organisms in the mid to upper reaches of the intertidal zone are also subjected to aerial exposure and exposed to the reduced salinity of a freshwater lens (freshwater overlying seawater with little or no mixing) for longer periods than individuals residing just above the subtidal zone.

Leptasterias aspera and L. epichlora are important intertidal predators in the Aleutian (Northern) zoogeographical province. Both species are members of the large, rather enigmatic L. hexactis species complex recently analyzed by Kwast et al. 1990, Stickle et al. 1992, Stickle and Foltz (1994), Foltz and Stickle (1994), Hrincevich and Foltz (1996), and Foltz (1998). In many areas along the Pacific coast of North America, L. hexactis strongly influences the community and population structure of intertidal zones. Competition for food (barnacles, limpets, and the bivalve Mytilus trossulus) produces complex patterns of spatial, temporal, and dietary overlap between sea stars (Menge 1972a, b). Intertidal areas may experience distinct salinity fluctuations that cause pronounced changes in the activity and physiology of L. hexactis (Shirley and
To analyze tidal effects of reduced salinity, *L. aspera* and *L. epichlora* were subjected to a simulated 30-10-30‰oS semidiurnal tidal cycle fluctuation; their perivisceral fluid osmolality and righting time as expressed by activity coefficient (Shirley and Stickle 1982b) was examined at regular intervals. To examine size effects within and between species, individuals from each species were separated into large and small size categories. The null hypotheses tested were: 1) perivisceral fluid osmolality and activity coefficients do not differ between *L. aspera* and *L. epichlora* when exposed to a simulated 30-10-30‰oS semidiurnal tidal cycle fluctuation; and 2) the distribution of large and small *L. aspera* and *L. epichlora* at Little Port Walter, AK does not differ between high, middle, and low intertidal zones.

**MATERIALS AND METHODS**

*Leptasterias epichlora* and *L. aspera* were collected on June 10, 1994 from Little Port Walter, Baranof Island, AK and transported to the laboratory at L.S.U. where they were maintained in 30‰oS seawater at 12°C. Little Port Walter is an estuary which displays high tidal amplitude and strong vertical salinity stratification due to the seasonal development of a freshwater lens system (Powers 1962).

Sea stars were collected from three vertically stratified areas within the intertidal zone: low (algal zone); middle (*Mytilus* zone); and high (*Fucus* zone). Standard horizontal starch-gel electrophoresis (Murphy *et al.* 1990) was performed on these individuals to confirm species identification using allozymic differences. A wide assortment of morphologic characters [wet weight (g), radius (length of longest ray = R in mm), interradius of the central disc (r in mm), color, ray shape, number of major and minor aboral pedicellaria, shape of aboral spines, abundance of major and minor adambulacral pedicellaria, number of spines per adambulacral plate, number of spines per carinal plate, and color of the podia] were recorded for each individual to establish a morphological means of species identification to be used in conjunction with fixed allele...
differences. A variety of both continuous (Tris citrate pH 8.0, amine citrate pH 6.9) and discontinuous (lithium hydroxide pH 8.3, TME 8.3) buffers were used to analyze four enzymes with diagnostic alleles (Foltz and Stickle 1994, Stickle et. al 1992). Arginine kinase (2.7.3.3), Peptidase-LA (3.4.13.11), Aconitase (4.2.1.3), and Superoxide dismutase (1.15.1.1) were examined for each individual and species identification was based on allele frequency differences.

Sea stars were subjected to a sinusoidal pattern of semidiurnal salinity fluctuation (30-10-30°oS), using an apparatus described in Stickle and Howey (1975). Individuals of each species (determined by morphological characters) were separated into large (≥8.5 g wet weight) and small (≤8.3 g wet weight) size classes to determine the effect of body size within and between species. Sixteen animals (eight small, eight large) from each species were analyzed at 0 hr to establish control values. Perivisceral fluid osmolality was recorded for the same sixteen individuals from each species every 3 hr over a 12 hr period. A 30-10°oS reduction occurred over a 5 hr period, followed by a 1 hr "slack" (stable salinity) period, and then the salinity increased back to 30°oS within 5 hr followed by a second 1 hr "slack" period.

Perivisceral fluid was removed from each individual by cutting off the tip of a single ray and extracting 10 μl via a micropipet. Six to 8 μl of perivisceral fluid was used to determine osmolality with a Wescor osmometer. Ambient water osmolality readings were also determined at each sampling time.

Following each three hour period, all sea stars were placed on their aboral side and righting response times were measured. Righting time was recorded as the time in seconds required for an individual to reach a vertical position after being placed on its aboral side on a horizontal surface under water (Shirley and Stickle 1982a). Twenty minutes (1200 seconds) was the maximal length of time allowed for righting. An activity coefficient was calculated for each individual by dividing 1000 by the righting
response time in seconds (Percy 1973). Individuals that did not right themselves in the time allotted displayed a minimal activity coefficient of 0.83.

**RESULTS**

Of 268 sea stars collected from the intertidal zone of Little Port Walter, species composition was 67.5% *L. epichlora*, 28.7% *L. aspera*, and 5.6% hybrids. *Leptasterias aspera* were larger in wet weight, length of the longest ray (R), and radius of the central disc (r) (Table 2.1). *Leptasterias aspera* were found more frequently in the high (*Fucus*) zone of the intertidal zone than was *L. epichlora*. Species composition did not vary between the low (26.3% *L. aspera*, n = 38) and middle zones (27.6% *L. aspera*, n = 29), but differed slightly in the high zone (36.6% *L. aspera*, n = 41). All hybrid individuals were found in the high (*Fucus*) zone, and the high zone *Leptasterias* contained a larger proportion of heterozygotes for the alleles analyzed. A size gradient was detected in the three zones; mean wet weight (± sd) of *L. aspera* was 8.85 ± 3.42 g from the high zone, 7.03 ± 3.49 g from the middle zone, and 5.41 ± 4.13 g from the low zone. Mean wet weight of *L. epichlora* was 6.19 ± 4.15 g from the high zone, 4.66 ± 1.57 g from the middle zone, and 3.61 ± 2.74 g from the low zone. Mean wet weight differences between high and low zone individuals of both *L. aspera* and *L. epichlora* are statistically significant at the p<0.05 level.

Perivisceral fluid osmolality did not differ between *L. aspera* and *L. epichlora* during the tidal cycle simulation. However, during the declining phase of the 30-10-30‰oS cycle, larger individuals of both species maintained higher perivisceral fluid osmolalities than small individuals (Figs. 2.1 and 2.2). During the increasing salinity phase, smaller individuals of both species demonstrated higher perivisceral fluid osmolalities than larger individuals. All individuals of both species remained slightly hypertonic to the ambient water with the exception of 3 hr (20‰oS) small *L. epichlora*. Activity coefficients (Fig. 2.3) were higher for small individuals than large individuals.
Table 2.1  *Leptasterias aspera* and *L. epichlora*. Morphological measurements of 268 sea stars from Little Port Walter, AK. Values for the small and large size classes collected from the intertidal zone are given for sea stars subjected to the 30-10-30%oS cycle only. Values listed are means ± SD.

<table>
<thead>
<tr>
<th></th>
<th><em>L. aspera</em></th>
<th><em>L. epichlora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wet weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>7.439 ± 8.830</td>
<td>4.761 ± 3.237</td>
</tr>
<tr>
<td>(range)</td>
<td>(1.785 - 28.164)</td>
<td>(0.322 - 22.925)</td>
</tr>
<tr>
<td>small</td>
<td>6.033 ± 2.022</td>
<td>4.818 ± 1.683</td>
</tr>
<tr>
<td>(range)</td>
<td>(2.444 - 7.872)</td>
<td>(2.185 - 8.230)</td>
</tr>
<tr>
<td>large</td>
<td>13.734 ± 4.403</td>
<td>11.389 ± 3.928</td>
</tr>
<tr>
<td>(range)</td>
<td>(8.687 - 28.164)</td>
<td>(8.817 - 22.925)</td>
</tr>
<tr>
<td><strong>Length of longest ray (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>32.844 ± 7.858</td>
<td>26.412 ± 7.074</td>
</tr>
<tr>
<td>(range)</td>
<td>(18.85 - 53.29)</td>
<td>(10.47 - 47.92)</td>
</tr>
<tr>
<td>small</td>
<td>27.749 ± 5.244</td>
<td>24.553 ± 2.952</td>
</tr>
<tr>
<td>(range)</td>
<td>(18.85 - 40.20)</td>
<td>(19.76 - 31.50)</td>
</tr>
<tr>
<td>large</td>
<td>38.724 ± 4.469</td>
<td>33.691 ± 4.477</td>
</tr>
<tr>
<td>(range)</td>
<td>(30.52 - 53.29)</td>
<td>(26.48 - 47.92)</td>
</tr>
<tr>
<td><strong>Radius of central disc (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>10.188 ± 2.161</td>
<td>9.174 ± 2.113</td>
</tr>
<tr>
<td>(range)</td>
<td>(6.12 - 17.94)</td>
<td>(3.80 - 17.06)</td>
</tr>
<tr>
<td>small</td>
<td>9.138 ± 1.257</td>
<td>8.954 ± 1.463</td>
</tr>
<tr>
<td>(range)</td>
<td>(6.80 - 12.06)</td>
<td>(5.75 - 12.52)</td>
</tr>
<tr>
<td>large</td>
<td>11.960 ± 1.860</td>
<td>11.387 ± 2.073</td>
</tr>
<tr>
<td>(range)</td>
<td>(9.25 - 17.94)</td>
<td>(7.86 - 17.06)</td>
</tr>
</tbody>
</table>
Figure 2.1 *Leptasterias aspera*. Perivisceral fluid and ambient water osmolality (mOsm·kg\(^{-1}\)) at each 3 hr interval during the 30-10-30%oS cycle. Values are means ± SE.

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Figure 2.2  *Leptasterias epichlora.* Perivisceral fluid and ambient water osmolality (mOsm•kg⁻¹) at each 3 hr interval during the 30-10-30%oS cycle. Values are means ± SE.
Figure 2.3  *Leptasterias aspera* and *L. epichlora*. Activity coefficient means (± SE) for small and large individuals at each 3 hr interval during the 30-10-30‰S cycle. All individuals at the 6 hr point (10‰S) and all *L. epichlora* individuals at the 3 hr point (20‰S) were unable to right themselves and displayed a minimal activity coefficient of 0.83.
for both species. However, most individuals of both small (6 of 8) and large (4 of 8) *L. aspera* were able to right themselves at the 3 hr (20%oS) point of the declining phase, whereas none of the *L. epichlora* individuals righted themselves at this stage of the experiment. No individuals were able to right at the 6 hr (10%oS) point. At 12 hours, all small *L. aspera* individuals were able to right, but only 4 of 8 large *L. aspera*, 5 of 8 small *L. epichlora*, and only 3 of 8 large *L. epichlora* could right themselves.

**DISCUSSION**

The effects of salinity on survival, feeding, activity, growth, and oxygen consumption in echinoderms have been well-documented (Drouin *et al.* 1985, Lawrence 1975, Sabourin and Stickle 1981, Shirley and Stickle 1982a, b, Stickle and Denoux 1976, and Stickle and Diehl 1987). Drouin *et al.* (1985) reported differences in population size structure of Gulf of St. Lawrence *Strongylocentrotus droebachiensis* exposed to fluctuating salinity, as compared to populations exposed to constant 30%oS, suggesting differential mortality as a function of size. Drouin *et al.* (1985) noted the absence of small *S. droebachiensis* individuals from the upper 2 meters of Caye a Cochons, a Gulf of St. Lawrence locality characterized by vertical salinity stratification, yet small individuals were found at similar depth at nearby sites which displayed homogenous ambient salinity. Himmelman *et al.* (1983) reported the absence of small *S. droebachiensis* in the upper regions of the St. Lawrence Estuary, and later (Himmelman *et al.* 1984) observed higher mortality rates in smaller individuals at locations with strong salinity fluctuations. These data suggest that the perivisceral fluid osmolality fluctuation of small *S. droebachiensis* is outside the range of capacity adaptation of this population.

Differences among populations in the salinity tolerance of several species of echinoderms, including *Leptasterias hexactis*, have been observed (Binyon 1961, Gezelius 1963, Sabourin and Stickle 1981, Shirley and Stickle 1982a, Stickle and Diehl
prompting these authors to suggest that physiological races of these species exist which are genetically or non-genetically adapted to reduced salinity. Exposure of *L. hexactis* and many other echinoderm species to reduced salinity for 7-28 days results in LC₅₀ values which do not change significantly with further exposure (Stickle and Diehl 1987). The rate of transfer to altered salinity does not affect tolerance in some echinoderms, as evidenced by LC₅₀ values. Results were similar for acute exposed and step-wise adapted individuals to altered salinity in *Asterias forbesi* (Loosanoff 1945) and *Strongylocentrotus droebachiensis* (Sabourin and Stickle 1981). Gezelius (1963) reported two physiological races of *Psammechinus miliaris* from the Swedish coast, a stenohaline population displaying a salinity tolerance from 30 to 34°/ooS, and a euryhaline population which tolerates 16 to 34°/ooS. Complete cross-acclimation between the two races occurs in 50 days, suggesting that the adaptation is not based on a genetic difference between the populations (Stickle and Diehl 1987).

The response of *L. aspera* and *L. epichlora* from Little Port Walter, AK to fluctuating salinity is affected by body size. This appears to be due to perivisceral fluid volume differences between small and large individuals. Because echinoderms typically lack complex osmoregulatory mechanisms, large individuals are better adapted to survive periods of low and decreasing salinity (sublethal stress) because they contain a higher volume of perivisceral fluid which does not turn over as quickly as the smaller volume of smaller individuals. This result may be due to a dampening effect of the body wall on changing ambient salinity (Stickle and Howey 1975). Larger individuals, however, experience a lag during periods of increasing salinity which may negatively affect activity and feeding rates.

Similar to results reported by Stickle and Howey (1975) in *Thais haemastoma*, the perivisceral fluid osmolality of *L. epichlora* was hyperosmotic to the ambient water osmolality throughout the increasing salinity phase of the simulated tidal cycle. Stickle
and Ahokas (1974, 1975) noted corresponding results from the echinoderms *Pisaster ochraceus* and *Cucumaria miniata*, and the mollusks *Katherine tunicata, Mopalia muscosa*, and *Thais lamelllosa*. Both size classes of *Leptasterias aspera* remained hyperosmotic to the ambient water throughout much of the increasing phase of the cycle, but became slightly hyposmotic to ambient water as it approached 30‰. These results conform to data on the perivisceral fluid from *Strongylocentrotus droebachiensis*; the echinoid displayed performance and activity drops as salinity decreased below 19‰ and failed to return to control values during the increasing phase of a 30-10-30‰ semidiurnal cycle (Stickle and Ahokas 1974). Data from Stickle and Denoux (1976) contrast with the results from *S. droebachiensis*. Stickle and Denoux (1976) observed an abundance of *S. droebachiensis* individuals from Lynn Canal, southeast Alaska, which apparently were adapted to low salinity. Stickle and Denoux (1976) attributed this discrepancy to size differences between these populations: *S. droebachiensis* from Lynn Canal, Alaska were substantially smaller (maximum size = 4 cm) than those from the Puget Sound, WA analyzed by Stickle and Ahokas (1974) (maximum size = 8 cm). Stickle and Denoux (1976) suggested a significantly slower turnover rate of perivisceral fluids in urchins of larger size.

At Little Port Walter, larger individuals of *L. aspera* are found more frequently than smaller individuals in the upper reaches of the intertidal zone where they are exposed to air and low salinity water for longer periods. This may represent a behavioral adaptation which, through conspecific (or congeneric with *L. epichlora*) competition, allows larger individuals access to food sources that smaller individuals may not be able to utilize. Because *L. aspera* individuals are generally larger than *L. epichlora* individuals, they are better adapted to the upper region of the intertidal zone. The perivisceral fluid osmolality of *L. aspera* varied less than that of *L. epichlora*, but this may strictly be a function of the larger size, on average, of *L. aspera*.
Activity coefficient data from this study correlates well with data from Sabourin and Stickle (1981), Percy (1973), and Lawrence (1975). Sabourin and Stickle (1981) observed similar activity values for *Strongylocentrotus droebachiensis* exposed to a 13°C, 30-10-30‰oS semidiurnal pattern of fluctuating salinity; Percy (1973) reported similar activity coefficients from *S. droebachiensis* at 15°C, 35‰oS, and Lawrence (1975) analyzed activity coefficients at different temperature and salinity combinations in the sea urchin *Lytechinus variegatus*. These studies indicate that activity coefficient determination is an accurate tool to assess the "functional well-being" of echinoderms and correlates well with other physiological indices. Mean activity coefficients were higher in *Leptasterias aspera* than *L. epichlora* during the declining phase of the tidal cycle, and *L. aspera* individuals recovered at a faster rate during the increasing phase. Activity coefficient differences suggest that large size may slow the righting responses of both *L. aspera* and *L. epichlora*, but this may be offset by the ability of large individuals to maintain higher perivisceral fluid osmolality during low tide periods and the ability to consume a greater size range of prey.

The upper (*Fucus*) area of the intertidal zone is a more variable environment than lower zones and individuals which inhabit this area are exposed to steeper environmental factor gradients. The decreased vulnerability to desiccation and thermal stress of larger *Leptasterias* individuals may be a result of behavioral adaptations producing vertical animal movement or selection within this zone.
EFFECTS OF FEEDING AND STARVATION ON ACTIVITY AND THE OXYGEN CONSUMPTION RATE OF LEPTASTERIAS SPP.

INTRODUCTION

The effects of feeding and starvation on energy budgets have been reported for a variety of species of marine invertebrates (Stickle 1971, Stickle 1985, Stickle and Duerr 1970, Wang and Stickle 1986). Feeding (consumption) is of primary importance among biotic factors affecting marine animals (Ivlev 1961). The feeding process relies upon a combination of physiological, behavioral, and morphological mechanisms, each of which is potentially affected by proximate environmental factors (Klinger et al. 1986). Consumption rates vary in response to environmental factors in several intertidal predators and represents a much more variable component of the energy budget than maintenance costs (Stickle 1985). Stickle (1985) reported that the primary determinant of scope for growth along environmental factor gradients was the large variation in ingestion rate compared with a relatively small variation in the rate of total caloric expenditure in five species of carnivorous marine invertebrates, including Leptasterias hexactis. Although there are a number of studies of nutritional processes in echinoids, there are few studies of the effects of feeding and starvation on asteroid echinoderms (Klinger et al. 1986). Seasonal variations in feeding rates and absorption efficiencies have been reported for the echinoids Strongylocentrotus droebachiensis (Lawrence 1975) and Lytechinus variegatus (Klinger et al. 1986), but, again, data on asteroid responses are lacking. Lawrence (1975) and Klinger et al. (1986) reported that feeding rates were adjusted over time to maintain similar rates of ingestion at lower temperatures. Miller and Mann (1973) suggested that feeding and absorption are strongly dependent on temperature, however others (Fuji 1967, Percy 1971) have suggested that seasonal variations in feeding processes are the result of physiological changes associated with other factors (reproductive events, increase in absorption...
efficiency). Stickle (1971) noted that biochemical and physiological studies should incorporate the reproductive state of the animal because reproductive cycles alter metabolism and seasonal differences in the available metabolic substrate may occur. Still others (Moore et al. 1963, Moore and McPherson 1965) have reported that while feeding rates may change seasonally, absorption efficiencies do not fluctuate seasonally.

Leptasterias spp. represent populations of widely-distributed, seasonally adapted intertidal invertebrates which compete for space and food resources with ecologically similar asteroids (notably Pisaster ochraceus and Evasterias troschelii). The extreme geographic range of this species complex dictates that tolerance to a broad spectrum of environmental factor gradients (including food availability) is essential to the fitness of the organism. Leptasterias spp. are distributed across two zoogeographic zones along the North American Pacific coast (the northern Aleutian zoogeographic province and the southern Oregonian zoogeographic province)(Foltz and Stickle 1994); these zones are characterized and delineated by pronounced faunal shifts across their boundaries. The null hypothesis tested was: nutritional state (fed, starved, starved/fed) does not influence the activity or oxygen consumption rates of Leptasterias spp.

MATERIALS AND METHODS

Leptasterias spp. were collected on August 31, 1994 from Little Port Walter, Baranof Island, Alaska (56°N 134°W) and flown to the laboratory at Louisiana State University where they were maintained at 12°C under constant illumination at 30‰oS for seven days. Prior to the experiment, sea stars were fed ad libitum on the blue mussel Mytilus trossulus. Individual Leptasterias were placed in perforated 710 ml plastic containers designed to permit water circulation. Containers were examined daily for dead specimens. Eight individuals were used per treatment/time combination and all individuals were analyzed for their rate of oxygen consumption and righting response.
The wet weight (g), R (length, in mm, of the longest ray), and r (mm radius of the oral
disk), of each sea star was determined at the start and end of each feeding experiment.

I. Ingestion Rate

Each sea star was initially provided with 8 mussels of edible sizes (total length
range = 9.4 - 32.9 mm, mean = 22.0 mm). Consumed mussels were measured to the
nearest 0.1 mm and replaced with similar sized mussels daily. The number of feeding
sea stars, the number of mussels consumed•sea star•day⁻¹, and the length of
consumed mussels were determined among sea stars which survived the experimental
period. The dry tissue weights of all consumed mussels from each treatment/time
combination were interpolated from a regression equation (y = 0.007x - 0.1; r² = 0.91)
determined from mussels (n = 31) of a similar size range to those consumed in the
experiment.

II. Oxygen Consumption Rate

Oxygen consumption rates were determined by flow-through respirometry as
described by Stickle et al. (1985) (Fig. 3.1). Twenty four individuals were sampled on
day 0 to establish control data. Sixteen individuals per treatment (A, B, and C) were
sampled on designated days throughout the experiment. Treatment A was fed
continuously and sampled on days 14 and 28. A starved group (treatment B) was
sampled on days 14 and 28, and a third group (treatment C) was starved for 14 and 28
days and then fed for 3 days prior to sampling (days 17 and 31).

III. Activity Coefficient

Activity was measured for each individual in each treatment group by recording
its righting response in seconds at the end of each feeding/starvation experiment.
Righting time was recorded as the time in seconds required for an individual to reach a
vertical position after being placed on its aboral side on a horizontal surface under water
(Shirley and Stickle 1982a). Twenty minutes (1200 seconds) was the maximal length

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Figure 3.1  Design of the flow-through respirometry apparatus.
of time allowed for righting. An activity coefficient was calculated for each individual by dividing 1000 by the righting response time in seconds (Percy 1973). Individuals that did not right in the time allotted displayed a minimal activity coefficient of 0.83.

IV. Statistical analysis

Dry weight of mussel tissue consumed, oxygen consumption, and activity coefficients were converted to weight-adjusted values for analysis of covariance and Tukey-Kramer adjusted least square means comparisons (SAS Institute, Inc. 1985). Interactions between the covariate (initial wet weight) and each treatment/time combination with ingestion rate (dry weight consumed), oxygen consumption, and activity were tested for significance.

RESULTS

Of 32 sea stars presented with prey, only one individual did not feed. Dry weight of mussel flesh consumed was highest in 28-day continuously fed animals (Fig. 3.2) and was significantly different (p<0.05) from other treatment/time combinations. Across all fed treatment/time combinations, females consumed significantly more (p<0.05) mussel tissue than males. Individuals starved 14 days and subsequently fed 3 days demonstrated a consumption rate (dry weight consumed/sea star/day) nearly as high as individuals which fed continuously for 14 days. Nearly all starved then fed treatment individuals fed upon the introduction of prey; however, 28-day starved, 3-day fed individuals consumed prey at rates substantially lower than those of 14-day starved, 3-day fed individuals.

Oxygen consumption rates (μL O₂·hr⁻¹·g⁻¹) were weight-adjusted to correspond to the sample mean wet weight (6.969 g) and differed between each treatment (Fig. 3.3); the control mean (20.53 μL O₂·hr⁻¹·g⁻¹) was higher than most experimental means. Among treatments, 28-day continuously fed individuals displayed the highest mean value (23.44 μL O₂·hr⁻¹·g⁻¹), followed by 14-day continuously fed individuals (20.26
Figure 3.2  
Dry weight of mussel flesh consumed *sea star\(^1\)-day\(^{-1}\) (mean ± SE) for each applicable feeding regimen. Treatment A (fed continuously 14 and 28 days); treatment C (starved 14 and 28 days then fed 3 days).
Figure 3.3 Weight-adjusted mean oxygen consumption rates ($\mu$LO$_2$·hr$^{-1}$·g$^{-1}$) for each feeding regimen. Treatment A (fed continuously 14 and 28 days); treatment B (starved continuously 14 and 28 days); treatment C (starved 14 and 28 days then fed 3 days). Values are means ± SE.
28-day continuously starved individuals (10.05 μlO₂·hr⁻¹·g⁻¹) had significantly lower (p<0.05) oxygen consumption than 28-day continuously fed individuals, but not significantly different from other treatments. Oxygen consumption rates (μlO₂·hr⁻¹) plotted against dry tissue weight (g) indicate a weight-specific increase in oxygen consumption with increasing tissue mass for each treatment/time combination (Fig. 3.4). Continuously fed (treatment A) and fed then starved (treatment C) individuals produced regression slopes which were significantly different within treatments, however, the slopes of 14- and 28-day continuously starved animals (treatment B) were not significantly different from each other. Intercept values differed significantly between 14- and 28-day continuously fed (treatment A) individuals and continuously starved (treatment B) individuals and 14-day starved, 3-day fed individuals (treatment C).

Activity coefficient means were highest (high = peak performance) among 14-day starved males (11.05) and lowest among 28-day starved males (3.79), but did not differ significantly between treatments or time (Fig. 3.5). Starvation for 14 and 28 days generated both the high and low mean activity values, and partially fed animals produced rates which were intermediate between 14 and 28 day continuously fed individuals. Continuously fed individuals displayed the highest mean change in wet weight (0.747 g) and were the only treatment group to show positive mean values in change in wet weight (Fig. 3.6). Continuously starved individuals showed a decrease in wet weight (-0.352 g) while starved then fed individuals also showed declines in wet weight (-0.148 g), but of a lesser magnitude.

**DISCUSSION**

Feeding rates and oxygen consumption rates of *Leptasterias* spp. from Little Port Walter, Alaska appear to be sensitive to the nutritional state of the animal. Feeding rates are clearly higher in animals provided with a continuous food supply,
Oxygen Consumption Rate (microliters O₂/hr)

Dry weight (g)

Figure 3.4  Oxygen consumption rates (μL O₂/hr⁻¹) for each feeding regimen plotted against dry tissue weight (g). Treatment A (fed continuously 14 and 28 days); treatment B (starved continuously 14 and 28 days); treatment C (starved 14 and 28 days then fed 3 days). Values are means ± SE.
Figure 3.5  Activity coefficients (mean ± SE) for each feeding regimen. Treatment A (fed continuously 14 and 28 days); treatment B (starved continuously 14 and 28 days); treatment C (starved 14 and 28 days then fed 3 days).
Figure 3.6 Change in wet weight (mean ± SE) for each feeding regimen. Treatment A (fed continuously 14 and 28 days); treatment B (starved continuously 14 and 28 days); treatment C (starved 14 and 28 days then fed 3 days).
but 14-day previously starved animals consumed food at rates similar to constantly fed animals over the 3 day feeding period. Animals starved for 28 days and then fed had low feeding rates, but survived the experimental period and began feeding when food was re-introduced. Starved animals had reduced oxygen consumption rates, relative to control and continuously fed animals, and rates of 28-day starved animals were significantly lower than those of continuously fed and 14-day starved animals.

Body size and feeding rates also influence oxygen consumption in *Leptasterias* spp. Larger *Leptasterias* consume more oxygen per g weight than smaller individuals and starved individuals have lower oxygen consumption rates than fed individuals. Continuously fed individuals of *Leptasterias* generally had the highest oxygen consumption and weight change. Continuously starved individuals had low levels of oxygen consumption and lost the largest amount of body mass. Continuously fed individuals thus had maximal growth and consumed more oxygen to maintain a higher metabolic rate. Starved treatment individuals decreased body mass and decreased standard metabolic activities. Increased metabolic rate, coupled with a decreased feeding rate, indicates a negative energy budget for individuals in these treatments. Although both starved and partially starved treatments (B and C) included the only individuals which did not survive the experimental period, the feeding and starvation data suggest that *Leptasterias* spp. from Little Port Walter can survive beyond 31 days with a negative energy budget.

Oxygen consumption rates of starved *Leptasterias* were similar to that of several pulmonate snails (von Brand *et al.* 1948, Duerr 1965) which demonstrated decreased oxygen consumption during starvation. Continuously starved *Leptasterias* decreased oxygen consumption rates between 14 and 28 days, however, starved then fed individuals showed increasing oxygen consumption rates between 14 and 28 days. Stickle and Duerr (1970) reported contrasting results from the prosobranch snail *Thaïs*
*lamellosa*. Oxygen consumption rates remained fairly constant or increased throughout a 53 day starvation period in *T. lamellosa*. Similarly, Stickle (1971) reported an increase in oxygen consumption during a 91 day starvation experiment in *T. lamellosa*. This physiological response was similar to *T. lamellosa* when starved at two different stages of the reproductive cycle and when the type of metabolic substrate was different (Stickle 1971). Coupled with an observed decrease in lipid stores during starvation, *T. lamellosa* appears to lack adaptations to decrease its metabolic rate under starvation (Stickle and Duerr 1970, Stickle and Bayne 1982).

The oxygen consumption rate of two scorpaenid fishes, *Scorpaena guttata*, a shallow-water species, and *Sebastolobus alascanus*, a deep-water inhabitant, differed between starved and fed treatments (Yang and Somero 1993). Oxygen consumption was 52% higher in fed *S. guttata* and 68% higher in fed *S. alascanus* than for starved individuals of the same species. Food availability is a primary factor in the depth-related reductions of metabolic rate (Yang and Somero 1993), and correlations between the respiratory rates of deep benthic animals and seasonal changes in surface productivity have been established (Smith and Baldwin 1984).

Food deprivation significantly lowers rates of oxygen consumption in poikilothermic animals (Beamish 1964). Several factors, including predator-prey interactions and food supply have been suggested as selecting forces for the reduction of metabolic rate (Yang and Somero 1993). The intertidal vertical distribution of prey, the density of prey, and the size of individual prey items are factors which influence oxygen consumption, and hence overall metabolic, rates of *Leptasterias*. Despite the limited respiratory mechanisms of echinoderms, *Leptasterias* can survive periods of nutrient depletion by decreasing oxygen consumption rates and lowering metabolic rates. Prey ingestion rates of *Leptasterias* spp. correlated well with data generated by Shirley and Stickle (1982a, b) from the broad taxon *L. hexactis*. Growth occurred among
continuously fed individuals which maintained high oxygen consumption rates. Starved individuals demonstrated decreases in weight and length measurements and subsisted on a negative energy budget. Starved then fed individuals fed quickly when presented with prey, and showed increases in oxygen consumption and tissue weights above the baseline levels established by continuously starved individuals. The null hypothesis was at least partially refuted. Feeding/starvation regimes significantly influenced ingestion rates and the oxygen consumption rate of *Leptasterias*, however activity coefficients were not significantly different between treatments in the study.
EFFECTS OF STEP-WISE ACCLIMATION AND ACUTE EXPOSURE TO TEMPERATURE IN SEASONALLY COLLECTED LEPTASTERIAS SPP.

INTRODUCTION

Gradual exposure to increasing or decreasing temperatures often produces a shift in the lethal minimum and/or maximum critical temperatures (Tc), as well as the preferred (optimal) temperature (To) of poikilotherms (Hazel and Prosser 1974). For most animals, particularly intertidal invertebrates, the limits of temperature tolerance change seasonally (Cloudsley-Thompson 1971). Intertidal invertebrates are exposed to diurnal, tidal, and seasonal variations in temperature (Shirley et al. 1978). High temperatures which are lethal to winter-acclimatized animals may fall within the capacity adaptation range of summer-acclimatized animals (Cossins and Bowler 1987). Similarly, low temperatures which are lethal to summer-acclimatized animals may also be within the functional range of winter-acclimatized individuals. Animal species which exhibit a wide geographical range may also show seasonal temperature tolerance differences between populations at the extremes of the range (Hochachka and Somero 1984). Indeed, it is the capacity and resistance adaptation limits which often dictate the geographical distribution limits of a species (Hardy 1978).

Temperature effects on invertebrate metabolism has received considerable attention (Hummel et al. 1997, Schmidt et al. 1992, Sommer et al. 1997, Farmanfarmaian and Giese 1963, Choe 1962, Fuhrman and Fuhrman 1959, Meyer 1935, and Koller 1930). Most of these studies have focused on oxygen consumption. For many mollusc, annelid, and echinoderm species, it has been shown that an increase in temperature leads to an increase in O2 consumption until a temperature threshold is exceeded, above which O2 consumption decreases (Kristensen 1983). Conversely, a few studies have shown that for some intertidal invertebrates the metabolic rate is independent of ambient temperature over a wide temperature range (Vernberg and...
Vernberg 1964, Newell and Pye 1970). Adaptations to the intertidal zone by some invertebrate species include an active metabolic rate which is temperature-independent (thermo-neutral) within the ambient or acclimation temperature range, and a temperature-insensitive standard metabolic rate (Newell 1969, 1976). Seasonal acclimatization and acclimation temperature may also greatly affect metabolic rate and range of the temperature-insensitive zone (Pye and Newell 1973).

Sommer et al. (1997) determined high and low critical temperatures for the annelid *Arenicola marina* and linked the onset of anaerobiosis to periods when the ambient temperature lies outside of aerobic threshold boundaries. In addition, Sommer and Portner (in review) have identified changes of 4°C, above their respective critical temperatures, as a marker point which induces non-acclimatory anaerobiosis and the presence of anaerobic metabolites in *A. marina* from North and White Sea populations. Sommer et al. (1997) hypothesize that at temperatures beyond the minimum and maximum Tc, energy demands of metabolism cannot be met by an adequate oxygen supply owing to insufficient capacity of circulatory and/or ventilatory systems. As a result, anaerobiosis is required to provide sufficient energy production. Within Tc values of *Arenicola*, intracellular pH of body wall musculature changes), but the pH-temperature relationship does not remain linear beyond the respective Tc’s (Sommer et al. 1997).

Typically, Tc’s vary among populations of the same species and during seasonal temperature acclimatization. Sommer and Portner (in review) observed lower Tc values in winter adapted *Arenicola marina* and in polar populations, as compared to summer adapted and temperate populations. Sommer and Portner (in review) relate this shift to increased mitochondrial density per cell and an increased oxidative capacity of individual mitochondria, and hence increased efficiency of aerobic energy production at low temperatures. The apparent lowering of the upper Tc during winter acclimation can be
attributed to Sommer et al.'s (1997) observation that latitudinal and/or seasonal temperature adaptation in *Arenicola* results in a parallel shift of both Tc values due to increased mitochondrial density in the summer, and therefore an increase in overall metabolic rate.

*Leptasterias* spp. represent populations of widely-distributed, seasonally adapted intertidal invertebrates. The wide geographic range of this species complex dictates that tolerance to a broad spectrum of temperatures is essential to fitness. *Leptasterias* spp. are distributed across two zoogeographic zones along the North American Pacific coast (the northern Aleutian zoogeographic province and the southern Oregonian zoogeographic province); these zones are characterized and delineated by a pronounced faunal shift across their boundaries. *Leptasterias* spp. from a winter-acclimatized Alaskan population show increased levels of oxygen consumption and decreased levels of feeding at temperatures above 15°C (Tamplin and Stickle 1998). Null hypotheses tested were: (1) temperature tolerance limits of *Leptasterias* spp. do not shift seasonally; (2) temperature does not influence ingestion rates, activity, or oxygen consumption rates in seasonally collected *Leptasterias* spp.; and (3) activity, feeding, and oxygen consumption rates do not differ in seasonally collected *Leptasterias* spp. when subjected to acute exposure versus step-wise acclimation to temperature.

**MATERIALS AND METHODS**

I. **Step-wise acclimation**

A. **Winter sample**

*Leptasterias* spp. were collected on November 12, 1995 from Little Port Walter (n = 120; water temperature = 1°C) and maintained at 5°C and 30‰/ooS. Annual water temperature minima (0°C - 1°C) occur between November - March; annual maximum water temperatures (12°C - 13°C) at Little Port Walter occur during July - August (Powers 1962). Individual *Leptasterias* were separated and placed into perforated
plastic containers which permit water circulation. Containers and 80 of the 120 total sea
stars were placed individually into 10 gallon aquaria (30°/ooS, 5°C) and step-wise
acclimated (2°C every two days) to one of five treatment temperatures (7.5°C, 10°C,
12.5°C, 15°C, 17.5°C). Sixteen individuals were analyzed per treatment temperature.
Sampling occurred after 7 (acclimatory period) and 21 (experimental period) days.

B. Summer sample

*Leptasterias* spp. were collected on September 15, 1996 from Little Port Walter
(n = 160; water temperature = 11°C) and maintained in the laboratory at 11°C and
30°/ooS. Individual *Leptasterias* were separated and placed into perforated plastic
containers which permit water circulation, then placed individually into 20 gallon
insulated aquaria (30°/ooS, 11°C) and step-wise acclimated (2°C every two days) to one
of eight treatment temperatures (5°C, 7.5°C, 10°C, 12.5°C, 15°C, 17.5°C, 20°C, and
22.5°C). Twenty individuals were analyzed per treatment temperature. Ten individuals
were sampled from each treatment on days 14 and 28.

II. Acute exposure

A. Winter sample

*Leptasterias* spp. were collected on February 27, 1998 from Little Port Walter (n
= 128; water temperature = 1°C) and maintained in the laboratory for 4 days at 5°C and
30°/ooS. Individual *Leptasterias* were then separated and placed into perforated plastic
containers which permit water circulation. Containers with sea stars were individually
placed into 20 gallon insulated aquaria at one of eight treatment temperatures (5°C,
7.5°C, 10°C, 12.5°C, 15°C, 17.5°C, 20°C, and 22.5°C). Temperature data were
recorded by an Onset Stowaway datalogger at 15 minute intervals throughout the 28-day
period to monitor thermal conditions in each tank. Daily running means ± SE for each
treatment tank were determined; 28-day means ± SE were: 5.11 ± 0.03°C, 7.37 ±
0.02°C, 10.44 ± 0.02°C, 12.54 ± 0.05°C, 15.22 ± 0.04°C, 17.14 ± 0.04°C, 20.94 ± 0.19°C, and 22.55 ± 0.09°C. Sixteen individuals were analyzed per treatment temperature. Eight individuals were sampled from each treatment on days 14 and 28.

B. Summer sample

*Leptasterias* spp. were collected on July 2, 1996 from Little Port Walter (n = 120; water temperature = 10°C) and maintained in the laboratory from 4 - 7 days at 10°C and 30‰ S. Individual *Leptasterias* were separated and placed into perforated plastic containers which permit water circulation. Containers with sea stars were individually placed into 20 gallon insulated aquaria at one of eight treatment temperatures (5°C, 7.5°C, 10°C, 12.5°C, 15°C, 17.5°C, 20°C, and 22.5°C). Fifteen individuals were analyzed per treatment temperature. Eight individuals were sampled from each treatment on day 14 and seven individuals sampled on day 28.

III. Tolerance Studies

Containers were examined daily and the number of surviving individuals were determined to calculate a 28-day LC₅₀ (the temperature at which half of the individuals die). Daily running temperature means were calculated and used to determine a trimmed Spearman-Karber LC₅₀ (Hamilton et al. 1977). Due to the high percentage of survivors at each temperature and the lack of complete mortality at the upper temperature (17.5°C), an LC₅₀ could not be calculated for the winter acclimation experiment. LC₅₀ data were generated from each of the other experiments. Non-overlap of 95% confidence intervals for LC₅₀ values was considered to be significantly different.

IV. Common methodology

All individuals were analyzed for initial and final wet weight, initial and final R, initial and final r, oxygen consumption rate, righting response, and amount of mussel tissue consumed. Ingestion rates were determined by initially providing each sea star with 8 mussels of approximately equal sizes (total range = 9.41 - 32.90 mm,
mean = 22.00 mm). Containers were examined daily and consumed mussels were measured to the nearest 0.1 mm and replaced with similar sized live mussels. The number of feeding sea stars, the number of mussels consumed•sea star•day^{-1}, and the length of consumed mussels were determined. The dry tissue weights of all consumed mussels from each treatment/time combination were interpolated from a regression equation [y = 0.007x - 0.100; r^2 = 0.91; where y = dry tissue weight (g) and x = mussel length (mm)] determined from mussels (n = 31) of a similar size range to those consumed in the experiment.

Oxygen consumption rates (µlO2•hr^{-1}•g^{-1}) were determined by flow-through respirometry as described by Stickle et al. (1985). Oxygen consumption rates were used to calculate temperature coefficient (Q_{10}) values for each 2.5°C temperature increment (Prosser and Brown 1961). Q_{10} represents the degree to which a metabolic rate process is affected by temperature. The Q_{10} varies greatly over different temperature ranges, even within similar biological systems. The Q_{10} for many physiological rates is typically 2 - 3, but may vary, particularly in invertebrate poikilotherms (Prosser and Brown 1961). High Q_{10} values (>3) indicate an increase in rate at the higher temperature or a reduction at the lower temperature beyond what would be expected from the natural slowing of physiological processes; low Q_{10} values (1 - 2) indicate temperature acclimation (Klinger et al. 1986).

An activity coefficient for each individual was derived by recording its righting response in seconds in each treatment group. Righting time was recorded as the time in seconds required for an individual to reach a vertical position after being placed on its aboral side on a horizontal surface under water (Shirley and Stickle 1982a). Twenty minutes (1200 seconds) was the maximal length of time allowed for righting. Activity coefficients were calculated for each individual by dividing 1000 by the righting
response time in seconds (Percy 1973). Individuals that did not right in the time allotted displayed a minimal activity coefficient of 0.83.

Dry weight of tissue consumed, oxygen consumption, and activity coefficients were converted to weight-adjusted values by analysis of covariance (ANCOVA) and Tukey-Kramer adjusted least square means comparisons (SAS Institute, Inc. 1985). Interactions between the covariate (initial wet weight) and each treatment/time combination with ingestion rate (dry weight consumed), oxygen consumption rate, and activity were tested for significance. Temperature coefficient ($Q_{10}$) values over 2.5°C temperature intervals were determined, and the significance of each was tested against the null hypothesis that $Q_{10} = 1.0$ using a modified $t$-test (Snedecor and Cochran 1971).

**RESULTS**

I. **Tolerance Studies (zone of resistance adaptation)**

The 28-day $LC_{50}$ from the summer acclimation experiment was 18.59 (95% confidence intervals: 19.61 to 16.87); the 28-day $LC_{50}$ from the summer acute exposure experiment was slightly, but not significantly, lower [16.62 (95% CI: 15.43 to 17.21)] (Fig. 4.1). 28-day $LC_{50}$ values were significantly lower in the winter acute experiment [8.75 (95% CI: 7.63 to 9.21)] than in summer collected animals. $LC_{50}$ values of summer acclimated and summer acute exposed animals converged after day 14 and remained stable for the remainder of the experimental period. The winter acute exposure experiment produced continually decreasing $LC_{50}$ values with the largest decreases evident on day 15 and day 20.

Winter acclimatized *Leptasterias* spp. that were step-wise acclimated to experimental temperatures (at 2°C per day) showed low mortality rates at most treatment temperatures, and no animals died at temperatures below 15°C over the 21-day period. Survivorship was 74% at 15°C and 81% at 17.5°C. No animals were exposed to 20°C or 22.5°C, temperatures which produce complete mortality in the acute transfer.
Figure 4.1  Daily LC₅₀ values (mean ± SE), for 28 days, of *Leptasterias* spp. as a function of acclimatization.
experiment. In contrast, winter acclimatized animals subjected to acute exposure to temperature displayed high mortality rates. All individuals exposed to 22.5°C and 20°C died within 3 and 4 days, respectively. Only 6 of 16 (37.5% survivorship) individuals survived 14 days at 17.5°C, and no animals survived beyond 26 days at 17.5°C. Ten of 16 (62.5%) individuals survived 14 days at 15°C, but none survived beyond day 20. Eight of 16 (50%) individuals survived 14 days at 12.5°C, but only one individual survived past day 16 and died on day 25. All individuals exposed to 5°C, 7.5°C, and 10°C survived 14 days, but three animals died at 10°C (18.75% survivorship) and 2 animals died at 7.5°C (12.5% survivorship) by day 28. No winter acclimatized individuals died at 5°C over 28 days in either experiment.

Survivorship of summer acclimatized and step-wise acclimated animals was 100% at 5°C, 7.5°C, 10°C, and 12.5°C for 28 days. A single individual (5%) died at 15°C (day 1), and 10 of 20 (50%) 17.5°C animals died within the 28 day period. All individuals exposed to 20°C died within 14 days, and all animals exposed to 22.5°C died within 11 days. Summer acclimatized animals showed higher mortality rates under acute exposure to temperature than under step-wise acclimation. Acute exposure to temperature produced higher survivorship in summer acclimatized individuals compared to winter acclimatized individuals. No animals died at 5°C, 7.5°C, 10°C, or 12.5°C during the 28 day period. Four of 15 individuals died at 15°C (73% survivorship), but these individuals survived to at least day 13. Five of 15 (66.7% survivorship) animals died at 17.5°C, three of which died within the first 7 days. All summer acclimatized individuals acutely exposed to 20°C and 22.5°C died within 5 days of exposure.

II. Consumption of prey (zone of capacity adaptation)

Temperature and length of exposure had significant effects (p<0.05) on feeding rates in each experiment, and the interaction of time and temperature was significant in both experiments utilizing summer acclimatized individuals, but not for the winter acute
exposure experiment. Feeding data for winter collected, step-wise acclimated individuals were collected at the end of the experimental period, and a temperature/time interaction could not be analyzed for this experiment. The wet weight of individual sea stars had a significant effect on dry weight of mussel tissue consumed (g tissue•sea star^-1•day^-1) in each of the four experiments.

A. Acute exposure, winter sample

Acute exposure to temperature in winter-acclimatized animals produced feeding rates (range 1.861 - 4.865 g tissue•sea star^-1•day^-1) similar to those of step-wise acclimated individuals. Among treatments, dry weights of consumed tissue•sea star^-1•day^-1 were highest in 14-day, 15°C animals (4.865 g), followed by 28-day, 7.5°C (7.783 g) and 10°C (4.623 g) individuals (Fig. 4.2A). Feeding rates varied between and among treatment temperatures, but were not significantly different between treatments.

B. Step-wise acclimation, winter sample

Feeding rates were decreased at high experimental temperatures (15°C and 17.5°C); mid-range (12.5°C and 10°C) temperatures stimulated higher rates but rates did not differ significantly from each other or the two higher temperatures; feeding was significantly (p<0.05) increased at the low temperature (7.5°C). The percent of sea stars which did not feed were 0% at 5°C (acclimatory period), 0% at 7.5°C, 6% at 10°C, 25% at 12.5°C, 75% at 15°C, and 50% at 17.5°C. Of the 24 individuals which did not feed, 7 died during the experimental period (4 at 15°C, 3 at 17.5°C). The average length of mussels available throughout the experiment (25.89 mm) did not differ statistically from the length of mussels consumed (23.77 mm). Among treatments, dry weights of consumed tissue•sea star^-1•day^-1 were highest at 7.5°C (3.021 g) and significantly different from the means of all other treatments (10°C =
Figure 4.2  Dry weight of mussel flesh consumed•sea star•day\(^{-1}\) (mean ± SE) for each time/temperature treatment combination of each experiment.

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2.014 g; 12.5°C = 1.771 g; 15°C = 1.069 g; 17.5°C = 1.293 g) (Fig. 4.2B). Dry weight consumed did not differ statistically among the other treatment temperatures.

C. Acute exposure, summer sample

Feeding rates of acutely exposed, summer acclimatized individuals were much lower than those of step-wise adapted, summer acclimatized individuals, but similar to experimental values of winter-acclimatized individuals. Significantly (p<0.05) decreased feeding rates (dry weights of consumed tissue*sea star−1•day−1) occurred at high temperatures (12.5°C, 15°C, and 17.5°C) in 14-day sampled individuals as compared to all other treatments, except 28-day, 17.5°C individuals (0.086 g) (Fig. 4.2C). 10°C, 28-day individuals displayed the highest mean feeding rate (4.156 g). The remaining treatment means ranged between 1.928 g and 2.870 g.

D. Step-wise acclimation, summer sample

Summer acclimated animals showed much higher feeding rates than winter acclimated animals across all treatment temperatures. Feeding rates varied between treatment temperatures and were generally higher at lower temperatures (5°C and 7.5°C) than at higher temperatures (15°C and 17.5°C). Feeding rates decreased between 14- and 28-day samples at each temperature (Fig. 4.2D). Intermediate temperatures produced values which varied within and among 14- and 28-day samples at most treatment temperatures. Nearly all animals fed at each temperature except 17.5°C, in which 15% did not feed. A single individual each (5%) did not feed in the 10°C and 15°C treatments. High experimental temperatures (22.5°C and 20°C) produced complete mortality within 6-14 days, but both temperatures initially stimulated feeding (tissue*sea star−1•day−1) before death.

III. Oxygen consumption (zone of capacity adaptation)

Temperature and length of exposure had significant (p<0.05) effects on oxygen consumption rates in each experiment. The interaction of temperature and time was
significant in summer acclimatized animals in both the acute exposure and step-wise acclimation experiments, but did not significantly affect winter acclimatized animals under either exposure regime. Wet body mass had a significant effect (p<0.05) on oxygen consumption rates (µLO₂·hr⁻¹·g⁻¹) in each of the four experiments.

A. Acute exposure, winter sample

Weight-specific oxygen consumption rates differed significantly (p<0.05) between 14-day, 17.5°C (66.26 µLO₂·hr⁻¹·g⁻¹) animals and all other treatments. No other treatments had values higher than 37.35 µLO₂·hr⁻¹·g⁻¹ (14-day, 15°C individuals) and most treatment means were below 29 µLO₂·hr⁻¹·g⁻¹ (Fig. 4.3A). Twenty-eight day, 7.5°C animals had the lowest value (17.25 µLO₂·hr⁻¹·g⁻¹). Oxygen consumption rates were not significantly different between 14- and 28-day animals within each treatment temperature, however only individuals maintained at 10°C or below survived for 28 days.

B. Step-wise acclimation, winter sample

Weight-specific oxygen consumption rates did not differ statistically between 7- and 21-day treatments at the same temperature, but differed significantly (p<0.05) between 7-day, 17.5°C animals and all other treatments. The 7-day, 17.5°C mean (53.92 µLO₂·hr⁻¹·g⁻¹) was 38% higher than in 21-day, 17.5°C individuals (39.01 µLO₂·hr⁻¹·g⁻¹) and 64% higher than the next highest temperature (7-day, 15°C; 32.91 µLO₂·hr⁻¹·g⁻¹). The oxygen consumption rate of 7-day, 17.5°C animals was over 100% higher than all other experimental means (Fig. 4.3B). Substantial increases in oxygen consumption at 17.5°C indicate higher metabolic rates at this temperature which appears to be above the zone of capacity adaptation of the Little Port Walter population as judged by mortality rates.
Figure 4.3 Weight-specific mean oxygen consumption rates (µlO₂·hr⁻¹·g⁻¹) for each time/temperature treatment combination of each experiment. Values are means ± SE.
C. Acute exposure, summer sample

Weight-specific oxygen consumption rates of individuals exposed to 17.5°C were significantly different from all other treatments. Fourteen-day, 17.5°C treated individuals were highest (61.01 μlO₂·hr⁻¹·g⁻¹); 28-day, 17.5°C treated animals had the lowest values (13.77 μlO₂·hr⁻¹·g⁻¹) (Fig. 4.3C). All other treatments displayed intermediate values ranging from 24.94 μlO₂·hr⁻¹·g⁻¹ (28-day, 10°C individuals) to 39.54 μlO₂·hr⁻¹·g⁻¹ (14-day, 15°C individuals).

D. Step-wise acclimation, summer sample

Weight-specific oxygen consumption rates differed significantly (p<0.05) between 14-day, 17.5°C treated animals (57.81 μlO₂·hr⁻¹·g⁻¹) and all but three other treatments [28-day, 12.5°C (49.53 μlO₂·hr⁻¹·g⁻¹); 28-day, 7.5°C (45.17 μlO₂·hr⁻¹·g⁻¹); and 28-day, 15°C (34.91 μlO₂·hr⁻¹·g⁻¹)] (Fig. 4.3D). Twenty-eight day, 17.5°C treated animals had the lowest value (17.43 μlO₂·hr⁻¹·g⁻¹). This differed (p<0.05) from the mean value for 14-day acclimated animals maintained at the same temperature. Oxygen consumption rates of sea stars increased between 14 and 28 days within treatment temperature, except the high (17.5°C) and low (5°C) extremes. Fourteen-day and 28-day 5°C treated animals had similar values, 32.14 and 28.55 μlO₂·hr⁻¹·g⁻¹, respectively.

IV. Temperature coefficient [Q₁₀] (zone of capacity adaptation)

A. Acute exposure, winter sample

Q₁₀ values differed (p<0.05) from 1.0 at the 15-17.5°C temperature increment (9.911) after 14 days and the 7.5-10°C increment (11.498) after 28 days. At temperatures below the 10 - 12.5°C increment, Q₁₀ values were not significantly different from 1.0 in winter acclimatized animals subjected to acute exposure to temperature (Fig. 4.4A). Q₁₀ values increased at higher temperatures in animals.

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sampled at 14 and 28 days; all individuals at temperatures higher than 10°C died by day 28.

B. Step-wise acclimation, winter sample

Q_{10} values for winter adapted animals subjected to step-wise acclimation were different (p<0.05) from 1.0 at the 12.5 - 15°C (7.717, 4.192) and 15 - 17.5°C (7.205, 5.784) increments for both 7 and 21 day samples, respectively (Fig. 4.4B). Q_{10} values for both 7 and 21-day animals at 7.5 - 10°C and 10 - 12.5°C increments were <2 and fell within normal, expected values. Q_{10} values decreased between 7 and 21 day individuals at all temperature increments.

C. Acute exposure, summer sample

Summer acclimatized animals subjected to acute exposure to temperature yielded low Q_{10} values (<2; none different from 1.0; p<0.05) at all temperature increments below 12.5°C (Fig. 4.4C). Individuals sampled at 14 days had an intermediate values (mean = 3.153) between 12.5 - 15°C temperature increment, which was significantly different from 1.0, as was the 15 - 17.5°C increment, which produced the highest Q_{10} value (5.671) in individuals sampled at 14 days. No individuals survived 28 days above 15°C.

D. Step-wise acclimation, summer sample

Q_{10} values were low (<2; none significantly different from 1.0) for each temperature increment in all animals sampled after 14 days, with the exception of the 15-17.5°C increment, which had the highest significant value (25.035) of any treatment/time combination in any of the experiments (Fig. 4.4D). Except for a relatively high value (6.264; significantly different from 1.0) at the 5 - 7.5°C increment, Q_{10} values were below 1, and not significant, for each temperature increment sampled after 28 days.
Figure 4.4  Temperature coefficient (Q₁₀) means (± SE) for each time/temperature increment of each experiment.  Q₁₀ values significantly greater than 1.0 are designated with an asterisk (*).
V. Activity coefficient (zone of capacity adaptation)

Analysis of covariance had significant (p<0.05) effects only between activity and temperature and activity and time treatment combinations in the winter acute experiment. Analysis of covariance did not reveal significant effects between activity and the covariate (initial wet weight) or temperature, nor were there significant interactions between temperature and time treatment combinations in any of the remaining experiments.

A. Acute exposure, winter sample

Activity coefficient means were highest among 14-day, 5°C and 7.5°C individuals (10.39), but did not differ significantly from other treatments, all of which displayed similar values ranging between 5.13 (14-day, 17.5°C animals) and 10.12 (14-day, 10°C individuals) (Fig. 4.5A). All individuals that survived the experiment righted themselves within the allotted 20 minutes.

B. Step-wise acclimation, winter sample

Activity coefficient means were highest among 7-day, 15°C individuals (15.98), but did not differ significantly from other treatments, including 21-day, 10°C individuals, which had the lowest mean activity coefficient (7.02) (Fig. 4.5B). All individuals righted themselves within the allotted 20 minutes.

C. Acute exposure, summer sample

Acute exposure to temperature in summer acclimatized Leptasterias spp. did not significantly affect activity coefficient values (range 4.354 - 12.528). Activity coefficients were similar to those in the other temperature experiments, and did not differ (t-test; SAS 1985) between or within treatments (Fig. 4.5C). No individuals that survived the experiment failed to right themselves.
Figure 4.5  Activity coefficient means (± SE) for each time/temperature treatment combination of each experiment.

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D. Step-wise acclimation, summer sample

Activity coefficient means were highest among 14-day, 5°C treated individuals (21.41), but did not differ significantly from other treatments, all of which displayed similar mean values ranging between 9.51 (14-day, 10°C animals) and 12.24 (28-day, 15°C individuals) (Fig. 4.5D). All individuals that survived the experiment righted themselves within the allotted 20 minutes.

DISCUSSION

Twenty-eight day LC₅₀ values were lower in winter acclimatized, acutely exposed *Leptasterias* spp. individuals (8.7°C) than in summer acclimatized animals (step-wise acclimation = 18.6°C; acute exposure = 16.6°C). LC₅₀ values of summer acclimated and summer acutely exposed animals converged after day 14, remained stable for the remainder of the experimental period, and by day 28 were not significantly different from each other. The winter acute exposure experiment produced continually decreasing LC₅₀ values. *Leptasterias* spp. from Little Port Walter are seasonally adapted to temperature and the maximum critical temperature (Tc) decreases 8-10°C during the winter months. These data indicate that a larger shift of Tc’s occurs seasonally in *Leptasterias* spp. from Little Port Walter than the 4°C shift observed *Arenicola marina* from the North (upper Tc = 25°C) and White Seas (upper Tc = 21°C) (Sommer *et al.* 1997). These data also indicate that both acute and gradual exposure to temperature induces thermal acclimation in *Leptasterias* spp., and may be a response to temperature fluctuations that occur both seasonally and diurnally in the intertidal zone.

For winter acclimatized individuals, mortality rates were much higher under acute exposure than under step-wise acclimation. Acute exposure to 20°C and 22.5°C resulted in 100% lethality within 4 days, and individuals maintained at these temperatures drastically reduced feeding rates over this 4 day period; acute exposure to 12.5°C, 15°C, and 17.5°C produced complete mortality between 14 and 28 days.
Mortality rates were low for step-wise acclimated, winter collected animals across all temperatures, but sea stars were not exposed to 20°C and 22.5°C, temperatures which produced complete mortality in all other experiments of this study. All individuals survived at temperatures below 15°C, and survivorship was high at 15°C and 17.5°C.

The reproductive state of *Leptasterias* spp. may have played a role in the determination of an LC₅₀ and mortality rates for winter acclimatized animals. The spawning and female brooding season of *Leptasterias* spp. begins in November and terminates in April (Chia 1966a, Menge 1975). The breeding season represents a period of forced starvation for brooding females and depletes energy reserves of both males and females (Menge 1975). *Leptasterias* spp. individuals from Little Port Walter that were collected on November 8, 1994 (pre-spawning) contained significantly more gonad and pyloric caecal tissue and possessed a lower percent body water than those collected on May 23, 1995 (post-spawning) (Tamplin, unpublished). Both male and female gonad indices were significantly higher in November (2.54 and 2.29) than in May (0.49 and 0.69), respectively. Nutrient reserves, as evidenced by pyloric caecal indices (total wet weight/ceacal dry weight x 100), were highest in winter collected females (7.76) and males (6.50) and lowest in spring collected individuals (females = 4.54; males = 4.19). *Leptasterias* spp. individuals used in the winter step-wise acclimation experiment were collected in early November, at the start of the reproductive season, when nutrient reserves are high. Because *Leptasterias* spp. individuals used in the winter acute experiment were collected in late February, at the end of the reproductive cycle, these individuals may have been more vulnerable to environmental stress, and hence demonstrated higher mortality rates.

In step-wise acclimated, summer acclimatized animals, complete mortality occurred within 14 days at 20°C and within 11 days at 22.5°C; only a single individual died at 17.5°C by day 14, but only one individual survived to day 28. One individual
acclimated to 15°C died on day 1, but the early onset of mortality suggests that this death was not related to temperature exposure. Mortality rates of summer acutely exposed individuals were similar to those of summer collected, step-wise acclimated animals.

Seasonal variations in feeding rates have been reported in a variety of marine invertebrates (Klinger et al. 1986, Stickle et al. 1985, Johns 1981a, b, Shirley et al. 1978, Lawrence 1975, and Percy 1971) although data on asteroids are limited (Shirley and Stickle 1982a). Klinger et al. (1986) reported that feeding rates of Lytechinus variegatus (seasonal temperature range 12°C - 31°C) varied significantly with temperature and were adjusted over time at a lower temperature to maintain rates of ingestion. Decreases in temperature would necessitate depression of kinetic energies associated with physiological systems, thus, control systems regulating ingestion in L. variegatus must compensate to maintain rates of ingestion despite temperature changes. Lawrence (1975) suggested that, although a sudden change in temperature will depress feeding rates of echinoids, rates of feeding are constant over temperature over long term periods. In contrast, Miller and Mann (1973) determined that feeding rates of Strongylocentrotus droebachiensis (seasonal temperature range 4-25°C) were temperature dependent. Percy (1971) observed depressed feeding rates in Strongylocentrotus droebachiensis at winter temperatures, but noted the amount of food absorbed is constant despite seasonal variation in temperature because absorption efficiency is higher at winter temperatures. Data of Percy (1971) partially correspond with that of Moore et al. (1963) and Moore and McPherson (1965), who reported that feeding rates and absorption efficiencies are constant over seasons in the echinoids Tripneustes esculentus and Lytechinus variegatus (seasonal temperature range 9-28°C). Moore and McPherson (1965) observed substantial annual variation in feeding rate, which complicated the relationship between temperature and feeding rate. Johns...
(1981b) reported variation in assimilation efficiency with temperature and higher efficiency values in *Cancer irroratus* (seasonal range 10°C - 23°C) larvae at both the high and low temperature extremes between 10°C and 30°C.

Consistent with data of Percy (1971) and Lawrence (1975) for *Strongylocentrotus droebachiensis*, but contrasting with data of Moore *et al.* (1963), and Moore and McPherson (1965) regarding *Lytechinus variegatus*, feeding rates of *Leptasterias* spp. from Little Port Walter, Alaska appear to be sensitive to elevated temperature regimes when previously acclimatized to annual winter minimal temperatures. Winter-collected, step-wise acclimated individuals exhibited reduced feeding rates at high temperatures (15°C and 17.5°C) and displayed the highest feeding rate at the lowest experimental temperature (7.5°C). Feeding rates (consumed tissue•sea star⁻¹•day⁻¹) of *Leptasterias* spp. from Little Port Walter are similar in magnitude with those reported for a variety of intertidal echinoderms (Klinger *et al.* 1986, Shirley and Stickle 1982a, Fuji 1967).

Acute exposure of winter acclimatized individuals produced feeding rates which varied within and between temperature treatments, and were highest at intermediate temperatures (7.5°C, 10°C, and 15°C). Unlike winter collected, step-wise acclimated individuals, acute exposure to 17.5°C did not decrease feeding rates in *Leptasterias* spp. These data contradict the findings of Klinger *et al.* (1986), who observed an increase in feeding rate of a Florida population of winter acclimatized *Lytechinus variegatus* acutely exposed to 16°C and 23°C. Both of these temperatures are within the normal winter environmental range for this species; temperatures which produce significantly decreased feeding rates in winter acclimatized *Leptasterias* spp. from Little Port Walter, however, lie outside of normal environmental winter temperatures.

Acute exposure of summer acclimatized animals significantly decreased feeding rates in *Leptasterias* spp. individuals sampled after 14 days at 12.5°C, 15°C, and
17.5°C, and individuals sampled after 28 days at 17.5°C. Feeding rates were elevated at low temperatures (5°C - 10°C) in animals sampled at 14 days, and in a broader range of temperatures (5°C - 15°C) in animals sampled after 28 days. Acute exposure to temperatures below the environmental summer norm (10°C - 12.5°C at Little Port Walter) initially stimulates feeding in summer acclimatized *Leptasterias* spp., and those below or slightly above the normal environmental summer temperature increase feeding rates between 14 and 28 days. Feeding rates of step-wise acclimated, summer acclimatized individuals were much higher than, but produced patterns somewhat similar to, those of the other temperature experiments. Step-wise acclimated, summer collected individuals demonstrated increased feeding rates at low temperatures (5°C and 7.5°C) and 12.5°C in animals sampled at 14 days. Animals sampled at 28 days showed decreased feeding rates, which more closely correlated with those from the other temperature experiments, across all temperatures.

Both temperature and time have a significant effect on the oxygen consumption rate of *Leptasterias* spp. from Little Port Walter. Oxygen consumption rates are significantly higher in both summer and winter acclimatized *Leptasterias* spp. when exposed to temperatures above the environmental maximum (12.2°C). In partial corroboration, Stickle and Bayne (1982) observed a significant effect of temperature on the oxygen consumption rate of the gastropod *Thais (Nucella) lapillus*, but reported no significant variation of oxygen consumption over time. In winter acclimatized, step-wise acclimated *Leptasterias* spp., high temperatures (15°C and 17.5°C) significantly increased oxygen consumption rates in animals sampled after 7 and 21 days. In winter acclimatized, acutely exposed individuals, oxygen consumption rates were significantly higher after 14 days at 17.5°C compared to all other temperatures, but decreased by day 28 and were not significantly different from other treatments. These data suggest that
acclimation to temperature occurs between 21-28 days in winter acclimatized *Leptasterias* spp from Little Port Walter.

Stickler and Bayne (1982) reported depressed oxygen consumption rate at 5°C, but not at 10°C, 15°C, or 20°C in *Thais (Nucella) lapillus*. Depression in oxygen consumption rate at 5°C was not observed in this study, and low temperatures did not significantly alter oxygen consumption rate in any of the experiments. However, the low temperature extreme of this study (5°C) does not approach the winter environmental low (0°C - 1°C) at Little Port Walter, and this temperature is probably above the summer lower critical temperature in *Leptasterias* spp. from Little Port Walter. Shirley *et al.* (1978) determined that seasonal changes in oxygen consumption rates occur in the gastropod *Littorina irrorata*. Shirley *et al.* (1978) noted increased oxygen consumption rates at 25°C (from February through April) and 30°C (from May through July) and attributed this to warm temperature stimulation of metabolism. An observed increase in oxygen consumption rate of winter acclimatized *L. irrorata* at 15°C may be indicative of cold temperature acclimatization. Oxygen consumption rates of *Leptasterias* spp. from Little Port Walter are similar in summer and winter acclimatized animals when exposed to temperatures that fall within the normal environmental temperature range. Oxygen consumption rates of *Leptasterias* spp. were significantly higher at 17.5°C in all experiments, suggesting that this temperature is above the capacity adaptation of this population.

Q10 values in the oxygen consumption rate of *Leptasterias* spp. were generally below 2 for each 2.5°C temperature increment between 5.0°C and 12.5°C in all experiments. Q10 values of all but summer acclimatized, step-wise acclimated individuals were high (>3) for the 12.5 - 15°C and 15 - 17.5°C increments, indicating that these temperatures lie at the upper end of the zone of capacity adaptation for *Leptasterias* spp.
Shirley et al. (1978) reported $Q_{10}$ values in *Littorina irrorata* that varied seasonally, producing low $Q_{10}$ values at low temperature increments (5°C - 10°C and 10°C - 15°C) during the winter months and similarly low $Q_{10}$ values at higher temperatures (15°C - 20°C) during the summer months. $Q_{10}$ values do not vary seasonally in *Leptasterias* spp., but consistently high $Q_{10}$ values at the 15°C - 17.5°C across all seasonal collections suggest that this temperature range is outside of the capacity adaptation of *Leptasterias* spp. from Little Port Walter.

Activity coefficients could not consistently be correlated with temperature or time in *Leptasterias* spp. from Little Port Walter. Activity coefficient means varied within and between temperature and time combinations in each experiment, but were not significantly different from each other for most treatment combinations. These data are in contrast to the findings of Kleitman (1941), Lawrence (1973, 1975), Percy (1973), Stickle et al. (1990), and Watts and Lawrence (1986) in several echinoid and asteroid species. Hagen (1994) could not correlate righting responses with temperature in *Strongylocentrotus droebachiensis*, and concluded that the righting response was not a valid, repeatable measure nor an accurate indicator of stress. Lawrence and Cowell (1996) subsequently demonstrated that the righting response of *Stichaster striatus* was a repeatable measure and indicative of organismal well-being. Activity coefficient values of *Leptasterias* spp. from Little Port Walter were similar in magnitude to those reported from other intertidal echinoderms (Lawrence 1973, 1975, Percy 1973, Stickle et al. 1990, and Watts and Lawrence 1986).

Activity coefficients of *Leptasterias* spp. from Little Port Walter, AK are highly variable upon both acute or acclimated exposure to temperature. Activity coefficients may not be a good indicator of thermal stress in *Leptasterias* spp. Activity coefficients were less variable at lower temperatures (5°C - 12.5°C), but in general were not statistically different from other temperature treatments with either acute or acclimated
exposure to temperature. Acute exposure to high temperatures (17.5°C - 22.5°C) produced greater variability in activity data, but not necessarily significantly different values among those individuals who successfully righted themselves within the allotted time period (1200 seconds). At high temperatures, some *Leptasterias* spp. individuals right quickly (<180 seconds), while others fail to right within 1200 seconds, indicating that physiological systems are highly variable on an individual basis at elevated temperatures.
CONCLUSIONS

Twenty-eight day LC$_{50}$ values were lower in winter acclimatized, acutely exposed *Leptasterias* spp. individuals (8.7°C) as compared to summer acclimatized animals (step-wise acclimation = 18.6°C; acute exposure = 16.6°C). LC$_{50}$ values of summer acclimated and summer acutely exposed animals converged after day 14 and remained stable for the remainder of the experimental period. The winter acute exposure experiment produced continually decreasing LC$_{50}$ values. *Leptasterias* spp. from Little Port Walter are seasonally adapted to temperature and the maximum critical temperature (Tc) decreases 8°C - 10°C during the winter months.

The reproductive state of *Leptasterias* spp. may have influenced the determination of an LC$_{50}$ and mortality rates for winter acclimatized animals. The spawning and female brooding season of *Leptasterias* spp. begins in November and terminates in April (Chia 1966a, Menge 1975). The breeding season represents a period of forced starvation for brooding females and depletes energy reserves of both males and females (Menge 1975). *Leptasterias* spp. individuals from Little Port Walter that were collected on November 8, 1994 (pre-spawning) contained significantly more gonad and pyloric caecal tissue and possessed a lower percent body water than post-spawning individuals collected on May 23, 1995 (Appendix). *Leptasterias* spp. individuals used in the winter step-wise acclimation experiment were collected in early November, at the start of the reproductive season, when nutrient reserves are high. Because *Leptasterias* spp. individuals used in the winter acute experiment were collected in late February, at the end of the reproductive cycle, these individuals may have been more vulnerable to environmental stress, and hence demonstrated higher mortality rates.

Acute exposure and step-wise acclimation to experimental temperatures (15°C and 17.5°C) above the normal environmental maximum (12.2°C) suppressed feeding and elevated oxygen consumption rates in *Leptasterias* spp. from Little Port Walter.
Increased metabolic rate, coupled with a decreased feeding rate, suggests a negative energy budget for individuals in these treatments. The temperature analysis suggests *Leptasterias* spp. exist near the upper limit of capacity adaptation when environmental temperatures reach the annual summer maximum (12.2°C), and undergo seasonal acclimatization to water temperature. Differences in the oxygen consumption rate of 14- and 28-day animals at 17.5°C indicate that 17.5°C is above the capacity adaptation of *Leptasterias* spp. from Little Port Walter. *Leptasterias* spp. respond to exposure to 17.5°C by initially increasing the oxygen consumption rate, and decreasing the feeding rate. By day 28, *Leptasterias* spp. display a negative energy budget and lowered oxygen consumption rate. *Leptasterias* spp. appears to be adapted to withstand sublethal levels of temperature shifts, which occur both seasonally and diurnally in the intertidal zone. *Leptasterias* spp. is adapted to survive short term periods of metabolic stress due to both food-deprivation and increased environmental temperature.

Individual size and consumption rates of prey influence oxygen consumption in *Leptasterias* spp. Larger *Leptasterias* consume more oxygen per g dry weight than smaller individuals and starved individuals have lower oxygen consumption rates than fed individuals. Continuously fed individuals showed maximal growth and consumed more oxygen. Starved treatment individuals decreased body mass and degraded tissue to maintain standard metabolic activities. Increased metabolic rate, coupled with a decreased feeding rate, suggests a negative energy budget for individuals in these treatments. While both starved and partially starved treatments contained the only individuals which did not survive the experimental period, feeding and starvation treatment group results suggest that *Leptasterias* spp. from Little Port Walter can survive beyond 31 days with a negative energy budget.

The perivisceral fluid osmolality of small and large *Leptasterias* spp. closely tracks the ambient water osmolality during simulated tidal cycles of salinity variation.
While minimal differences were evident between *L. epichlora* and *L. aspera* when exposed to fluctuating salinity, a size-related response was detected in both species. Larger individuals contain a larger volume of perivisceral fluid which resists changes in osmolality, and hence produces a slower turnover rate of fluid. Larger *Leptasterias* spp. individuals may show reduced vulnerability to low salinity during tidal fluctuations of salinity, and consequently be better adapted to inhabit the upper intertidal zone in areas that develop seasonal freshwater lenses.

In contrast with the findings of Kleitman (1941), Diehl *et al.* (1979), Ellington and Lawrence (1974), Himmelman *et al.* (1984), Lawrence (1975), Lawrence and Cowell (1996), Percy (1973), and Stickle *et al.* (1990), activity coefficients did not correlate with temperature gradients or nutritional state of the animal. Activity coefficients were not significantly different between feeding treatments or time in the nutritional study, and no consistent patterns in activity coefficient data were observed relative to temperature exposure and time in *Leptasterias* spp. from Little Port Walter. However, salinity fluctuation directly affected activity; reduced salinity increased righting times and negatively impacted activity in *Leptasterias* spp. A relationship of activity coefficient with salinity is consistent with results reported by Diehl *et al.* (1979) for the asteroid *Luidia clathrata*, Himmelman *et al.* (1984) for the echinoid *Strongylocentrotus droebachiensis*, and Shirley and Stickle (1982a) for *Leptasterias hexactis*. 

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BIBLIOGRAPHY


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APPENDIX

Seasonal pyloric caecal and gonad indices and percent body water for male and female *Leptasterias* spp. from Little Port Walter, Alaska. Individuals were collected on: August 31, 1994 (Summer); November 8, 1994 (Winter); and May 23, 1995 (Spring).

<table>
<thead>
<tr>
<th>Season/Sex</th>
<th>Gonad Index</th>
<th>Caecal Index</th>
<th>% Body Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer Females</td>
<td>1.065</td>
<td>5.911</td>
<td>73.126</td>
</tr>
<tr>
<td>Summer Males</td>
<td>1.022</td>
<td>5.577</td>
<td>73.709</td>
</tr>
<tr>
<td>Winter Females</td>
<td>2.289</td>
<td>7.756</td>
<td>74.591</td>
</tr>
<tr>
<td>Winter Males</td>
<td>2.542</td>
<td>6.497</td>
<td>75.422</td>
</tr>
<tr>
<td>Spring Females</td>
<td>0.688</td>
<td>4.654</td>
<td>76.080</td>
</tr>
<tr>
<td>Spring Males</td>
<td>0.497</td>
<td>3.860</td>
<td>76.485</td>
</tr>
</tbody>
</table>
VITA

Jeffrey W. Tamplin was born in Wareham, Massachusetts, on September 20, 1964. He earned his bachelor of arts (A. B.) degree from Augustana College, Rock Island, Illinois, in May, 1986. While at Augustana, he was selected by Dr. William Hammer to participate in a National Science Foundation sponsored paleontological trip to Antarctica during the austral summer of 1985-86 which resulted in the discovery of several new species of labyrinthodont amphibians and synapsid reptiles.

In August of 1986, he enrolled as a graduate student at Louisiana State University, Baton Rouge, Louisiana, and earned his master of science (M. S.) degree from L.S.U. in August 1988. He was hired thereafter to teach and coordinate the Introductory Zoology labs for the Department of Zoology and Physiology at L.S.U. In August 1989 he was additionally hired to teach Introductory Biology I and II through L.S.U.'s Evening School and intersession program. He held these positions throughout his career at L.S.U. In 1990, he was selected to participate in a second N.S.F. trip (1990-91) to Antarctica which resulted in the discovery of the first known dinosaur fossils from the continent.

In August 1993, he entered a doctoral program through L.S.U.'s Department of Zoology and Physiology under the auspices of Dr. William B. Stickle. In 1994, he was selected to teach in the College of Basic Sciences Medical College Admissions Test (MCAT) preparation course, and has continued to teach the course each time it was offered. During the years 1993, 1995, 1996, 1997 he was named in the top 10% of instructors in the College of Basic Sciences based on teaching evaluations and performance. Jeff earned a 10-year university Service Award in August 1998. Jeff has been a member of the American Society of Zoologists since 1993 and was inducted as a full member of Sigma Xi Scientific Research Honor Society in 1998. Jeff will graduate with the degree of Doctor of Philosophy in zoology in May, 1999.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Jeffrey William Tamplin

Major Field: Zoology

Title of Dissertation: Effects of Fluctuating Salinity, Nutritional State, and Temperature on Leptasterias spp. from Little Port Walter, Alaska

Approved:

[Signatures]

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

December 4, 1998