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Effects of seasonal variation in water and air temperature on the righting response of *Leptasterias* spp. from the west coast of North America

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ABSTRACT: Seasonal variation in water temperature is 6–13°C at San Juan Island, WA and 1–15°C at Sunshine Cove, Ak and air temperature ranges are below 0°C to 32°C on San Juan Island and to 27°C at Sunshine Cove. *Leptasterias* spp. from San Juan Island, WA in September exhibited a 28 day LC_{50} of 20.5°C, activity coefficients did not vary significantly at temperatures between 12–18°C but significant reductions occurred at 27 and 30°C. *L.* spp. from Sunshine Cove, AK; their activity coefficients varied significantly over 7 days at 10–19°C in August, and at 4–19°C in December. The activity coefficient did not vary during 3 h aerial emersion at 10 or 15°C but was depressed at 19°C. *L.* spp. are well adapted to the seasonal water temperature range but may be stressed during summer aerial exposure. Lactic acid accumulated in a non-significant manner during aerial exposure and in anoxic seawater.

1 INRODUCTION

The rocky intertidal zone on the Pacific coast of North America is one of the most physically complex and variable environments on earth. Seasonal changes in seawater temperature ranged from 12.1 to 15.4°C from 1983 to 1993 at Monterrey, CA (Barry et al., 1995), from 6–13°C in the San Juan Islands, WA (Stickle, 1970) and from 1–15°C at Sunshine (Ravioli) Cove along the Lynn Canal North of Juneau, AK(Stickle, 1970, Stickle and DeNoux, 1974). Air temperatures can reach 32°C during daytime tidal emersion in the San Juan Islands and 27°C at Ravioli Cove (personal observations). Air temperatures during night time aerial exposure during the winter at both locations can fall below 0°C.

Activity coefficients, as an index of their righting response A.C. = 1000/righting time in seconds), have been used as a sensitive behavioral indicator of the functional well being of echinoderms along environmental stressor gradients. Activity coefficients were determined to be repeatable measures when determined under constant conditions over four consecutive days in *Stichaster striatus* and to decrease significantly following 8 h 10 min emersion at 15–18°C Lawrence and Cowell 1996. Temperature and salinity affected the activity coefficient and growth rate of *Luidia clathrata* after 30 days exposure and the activity coefficient appeared to be related to decreased food intake and an apparent increase in the metabolic cost of cell volume regulation (Watts and Lawrence 1990). Activity coefficients have also been utilized as an indicator of echinoderm stress as a function of salinity gradients (Stickle and Diehl 1987). The activity coefficient of *Leptasterias hexactis* varied directly with ambient salinity (Shirley and Stickle 1982a) and covaried with variation in the energy budget of that species (Shirley and Stickle 1982b).

The normoxic aerobic rate of heat production accounts for all of the total heat production of the asteroid *Asterias rubens* (Grainger 1968). Because of poor perfusion of the perivisceral cavity of *Strongylocentrotus droebachiensis* during maximum ovarian development, 76–92% of heat dissipated is from anaerobic energy metabolism, with the production of lactate accounting for 37% of the total anoxic heat dissipation, which suggests that other end products of anaerobiosis may also be present (Bookbinder and Shick, 1986). The predominant glycolytic end product under potentially anaerobic metabolic conditions, such as environmental anoxia or aerial exposure, is lactic acid since opine dehydrogenases are essentially absent from the higher marine invertebrate phyla (Arthropoda (Crustacea) and Echinodermata) (Livingstone et al. 1983, 1990). Therefore, we assayed for the presence of L-lactic acid as an index of anaerobic stress in *Leptasterias* spp. during tidal emersion and during exposure to anoxic seawater.

This research project was designed to determine the temperature tolerance of a cryptic species complex of *Leptasterias* spp. (Flowers and Foltz, 2001) collected from Mitchell Bay, San Juan Island, WA during early

September, 1998 and compare their temperature tolerance with their righting response, expressed as activity coefficients (Lawrence and Cowell, 1996) as a function of seawater temperature. Seasonal change in the activity coefficient of

Leptasterias spp. collected from Sunshine Cove (Ravioli Cove) along the Lynn Canal north of Juneau was determined over a week in August and December 1998 at a series of ambient water temperatures. The effects of three hours aerial exposure at 9, 15, and 19°C for sea stars collected from Lena Cove, AK (~12 km south of Sunshine Cove, AK during August 2005. Finally, lactic acid accumulation was monitored as a function of duration of exposure to anoxic seawater and aerial exposure to determine a potential switch to anaerobic end product production as a function of glycolysis in *L.* spp.

2 MATERIALS AND METHODS

Leptasterias spp. were collected at Mitchell Bay, WA (48°33.2′N; 123°10.2′W) on September 2, 1998 and flown to LSU where temperature tolerance experiments were conducted in a recirculating seawater system over a 28 day time period at 12, 15, 18, 21, 24, 27, and 30°C. Sample size was 11 sea stars per experimental temperature. Activity coefficients were performed for each sea star 6 hours after introduction to experimental temperatures during the 28 day experiment (AC = 1000/ righting time in seconds (1200 sec maximum).

Sea stars were collected from Sunshine Cove (Latitude 58°30.8′N; Longitude 134°55.8′W), North of Auke Bay, AK on August 23 and December 14, 1998. Sea stars were returned to the NMFS laboratory where activity coefficients were determined at 0, 2, 4, 12, 24, 48 and 168h at several environmental temperatures in temperature controlled flow through seawater systems. The usual sample size was 8 sea stars per temperature-time combination. *L.* spp. were also transferred from 4, 9, 14, and 19°C experimental flow through systems to the 24°C system four days after the start of the initial experiment in December and survival and activity coefficients were determined at the same time intervals. A total of 20 sea stars were transferred from each experimental temperature system and activity coefficient determinations were made on 8 sea stars at each temperature-time combination.

Seastars were collected on August 15, 2005 at Lena Cove, AK (58°23.5′N; 134°44.7′W), returned to the NMFS Auke Bay Laboratory and subjected to either a simulated 3h emersion at 9, 15, and 19°C or to anoxic 30 PSU seawater for 30, 60, 90 and 120 minutes. The maximum Po_2 of the seawater during the anoxic exposure experiments was 9 Torr. Activity coefficients were determined prior to and after the 3 h aerial exposure period. Sample size was 8 sea stars per experimental determination.

Sea stars were flash frozen on dry ice, stored in a −80°C ultracold freezer then shipped to Louisiana State University in a liquid nitrogen shipper and stored in an ultracold freezer until homogenization. Sea stars were weighed and homogenized in 10 times their wet weight (g) in ice cold perchloric acid (0.6 mol/L). The homogenate was centrifuged for 15 minutes at 3000 g at 2°C. The supernatant (4 ml) was transferred to a fresh tube where 0.01 ml methyl orange (0.05%, w/v) was added. The supernatant was neutralized by titration with 3M KOH. A 0.2 ml aliquot of the neutralized supernatant was used for the assay sample. L-lactic acid assays were determined by the Franz Noll procedure (Bergmeyer, 1983). The final assay contained: 116 mmol/l glutamate, 0.93 mmol/l NAD, 1.3 kU/l ALT (GPT), and 4 kU/l LDH. L-lactic acid was used as the standard for this assay. The assay absorbance was read at 340 nm on a spectrophotometer after 2 h incubation at 25°C.

ANOVA analysis and Turkey's tests were performed on activity coefficients if they were normally distributed. Student's t-tests were performed on the activity coefficients of sea stars prior to and after emersion. In some cases data did not meet the underlying assumptions of ANOVA. We also conducted non-parametric ANOVA's using permutation according to the methods of Anderson and Ter Braak (2003). In all cases parametric and non-parametric analyses agreed regarding the significance of variance components indicating that violations of assumptions were not causing the observed significant differences. Herein we report only the parametric results. The 28 day LC_{50} was determined by the Spearman Karber method.

3 RESULTS

The 28 day LC_{50} of *Leptasterias* spp. collected at Mitchell Bay, WA on September 6, 1998 was 20.5 ± 0.3°C (95% C.I.). The LC_{50} declined from 25.46°C on day one to 20.5 ± 0.3°C on day 6 of exposure and did not change through day 28 of the experiment. The water temperature at the time of collection was 13.2°C. Activity coefficients of *L.* spp. are significantly different across the temperature range of 12–30°C on day 1 of the 28 day experiment (One way ANOVA; $p < 0.0001$). Tukey's Post ANOVA analysis of all pairwise comparisons indicates that the activity coefficient of *L.* spp. held at 12–21°C were not significantly different from each other or from those maintained at 24°C. Activity coefficients of *L.* spp. maintained at 27 and 30°C were significantly lower than those of sea stars exposed at 12–21°C but were not significantly lower than the activity coefficients of sea stars maintained at 24°C (Table 1). Furthermore and importantly the percent of the 11 sea stars not righting in 20 min was 18.2, 63.6, and 50 at 24, 27, and 30°C. Five of the 11 sea stars held at 30°C died prior to the determination of activity coefficients one day after introduction to that experimental treatment.

The activity coefficient of *L.* spp. collected from Sunshine Cove, AK. on August 6, 1998 varied significantly as a function of temperature ($P < 0.0001$) and time ($p < 0.0364$) but not their interaction (Fig. 1A).

Table 1. Activity coefficients of *Leptasterias* spp. collected from Mitchell Bay, WA and subjected to an experimental water temperature gradient are given as $x \pm 0.3$°C Percent not righting in 1200 seconds out of number tested is given in the column labeled Percent.

°C	x ± SEM	Tukey's test	Percent not righting
12	9.75 ± 5.13	A	0
15	6.64 ± 3.19	A	0
18	9.93 ± 4.01	A	0
21	6.19 ± 2.29	A	0
24	5.83 ± 4.25	AB	18
27	1.81 ± 1.49	B	64
30	3.50 ± 3.31	B	50

Likewise there was significant variation as a function of time at 19°C ($p < 0.0081$). Activity coefficients after 2 and 4 h exposure were significantly lower than activity coefficients after 168h exposure ($p < 0.05$), indicating a shock response to elevated water temperature during the summer.

The activity coefficient of L. spp. collected from Sunshine Cove, AK.on December 14, 1998 varied significantly as a function of temperature ($p < 0.0001$) and time ($p < 0.284$) but not their interaction (Fig. 1B). Activity coefficients were significantly higher at 14°C than at 9°C and activity coefficients at these temperatures were significantly higher than those observed at 4 and 19°C (Tukey's test). Sea stars did not right themselves in 20 minutes at 24°C at 2, 4, and 12 h, and all were dead at 24 h. There was no significant variation in the activity coefficient of *L.* spp. over the week of the experiment at 4, 19, or 19°C but the activity coefficient of sea stars maintained at 9°C varied over time ($p < 0.0340$) but Tukey's test did not vary significant variation in the activity coefficients tested over the experimental duration. None of the *L.* spp. transferred from the 4, 9, 14, or 19°C experimental systems survived 48 h exposure to 24°C seawater. Activity coefficients were minimal at 2, 4, 12, and 24 h and more than 50% of the sea stars from all temperature groups were dead by 24 h.

Three hours of aerial exposure in August had no effect on the activity coefficient of *L.* spp. prior to and after emersion at 9°C where the pre and post-exposure activities were 8.97 ± 4.94 and post-exposure activities were 7.41 ± 3.30(t-test $p > 0.4361$) or 15°C where the pre and post-exposure activities were 10.90 ± 6.30 and 5.19 ± 2.19 (t-test $p > 0.0707$). However, the activity coefficient of *L.* spp. exposed to emersion at 19°C for three hours declined from a pre-emersion value of 9.46 ± 2.70 o a post emersion value of 1.60 ± 1.80 (t-test $p > 0.0003$). Six of eight sea stars tested did not right themselves after 20 minutes (A.C. = 0.83) in the 19°C tidal emersion experiment.

Lactic acid was present in all sea star treatment groups that were sampled during the emersion experiments at 9, 15, and 19°C but the accumulation of lactic acid was variable and ANOVA analysis indicated non-significant temperature and time effects. Treatment

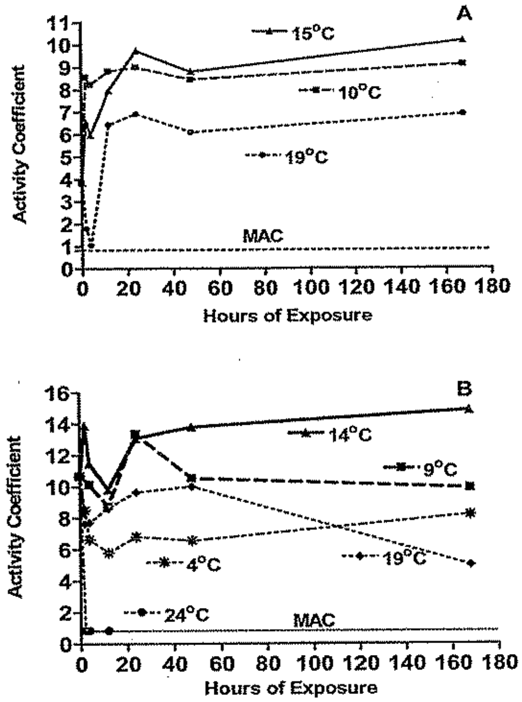

Figure 1. Activity coefficients of *Leptasterias* spp. collected on August 6, 1998 (A) and on December 14, 1998 from Sunshine Cove, AK and subjected to a series of experimental temperatures relative to the seawater temperature at the time of collection. MAC is the minimum activity coefficient (0.83) determined for sea stars that were alive but did not right themselves in 1200 seconds (20 minutes) elapsed time.

means (±SE) varied between 36 ± 29 (6) μM lactic acid/g wet^{-1} at 19°C before emersion and 372 ± 247 (7) μM lactic acid/g wet^{-1} after 3 h emersion at 15°C.

L. spp. accumulated lactic acid in a time dependent manner over 2 h exposure to anoxic seawater at 9, 15, and 19°C (ANOVA $p < 0.003$) but the temperature effect was not significant. Treatment mean values increased linearly at 9 and 15°C but one way ANOVA analysis at each temperature did not indicate a significant time effect with respect to anoxic exposure. Treatment mean values varied between 36 ± 29 (6) μM lactic acid/g wet^{-1} at 19°C in normoxic seawater and 1808 ± 700 μM lactic acid/g wet^{-1} after 2 h exposure to anoxic seawater at 15°C.

4 DISCUSSION

sThe activity coefficient of *Leptasterias* spp. collected at Mitchell Bay, WA in September, 1998 was not a sensitive indicator of seawater temperature between 12 and 21°C while a percentage of sea stars not righting after 20 minutes was elevated at 24°C (18.2%), 27°C (64%), and 30°C (50%). While activity coefficients were determined one day after exposure to each temperature treatment the 28 day water temperature LC_{50} was 20.5 ± 0.3°C. Therefore, determination of the ability of sea stars to right in 20 minutes proved to be a good indicator of the 28-day temperature zone of resistance adaptation of *L.* spp. to water temperature. If a significant percentage of sea stars can't right in 20 minutes at an experimental temperature in short term exposures, that experimental temperature likely falls outside the zone of capacity adaptation for the species at that location and will fall in the zone of lethality.

Seawater temperature affected the activity coefficients of L. spp. at the Sunshine Cove, Ak. location. The righting response (activity coefficients) of *L.* spp. collected during the summer from Sunshine Cove, Ak and exposed to 10 or 15°C seawater in the lab did not vary over time but the activity coefficient declined after one week exposure to 19°C seawater. The seasonal change in water temperature is −1 to 15°C. Sea stars collected in December exhibited significantly reduced activity coefficients at 4 and 19°C compared to those at 9 and 14°C over the course of one week. *L.* spp. did not right themselves in 20 min at 24°C at 2, 4, and 12 h and all were dead by 24 h exposure. Sea stars do not tolerate 24°C seawater at any time during the year. Seasonal changes in water temperature are not very large on the west coast of North America, being only 3.3°C at Monterrey, CA (Barry et al. 1995), 7°C on San Juan Island, WA (Stickle, 1970) and 14°C at salinity stratified Sunshine Cove, AK Stickle and DeNoux, 1974) in contrast to a greater seasonal change in water temperature in Barataria Bay, LA where the monthly average seasonal water temperature range was 17.6°C (Barrett, 1971).

Aerial emersion for three hours typical of mid-tidal *L.* spp. had no effect on their activity coefficient at 9 or 15°C but exposure at 19°C significantly reduced the sea star activity coefficient. It is important to note that *L.* spp. are found on under rocks and in crevices during aerial exposure during the daytime low tides during the summer in their microhabitat. Menge (1972) observed *L.* spp. to be actively foraging at night but not during the day on San Juan Island, WA. The foraging behavior of *L.* spp. makes them more susceptible to freezing during the nocturnal low tides during the winter than during the daytime low tides experienced during the summer in Washington and Alaska. The righting time and activity coefficient of *Stichaster striatus* collected from the coast of Chile and subjected to 8 h 10 min of emersion at air temperatures reaching 18°C during the day was significantly reduced when compared with these measures of activity as an indication of sublethal stress after re-immersion (Lawrence and Cowell 1996). The emersion time of *S. striatus* was much longer than the expected emersion time at their tidal level.

Lactic acid accumulated in *L.* spp. during aerial exposure and exposure to anoxic seawater but in a non-time course manner. This indicated that the sea stars exhibit a degree of anaerobic metabolism under these conditions. Lactic acid accumulates in the gonads and perivisceral fluid of the sea urchin *Strongylocentrotus droebachiensis* when they had a maximal ovarian index as a result of poor perfusion of the ovaries (Bookbinder and Shick, 1986). The high permeability of the integument of *L.* spp. likely accounts for the lack of a time course dependent accumulation of lactic acid during aerial and anoxia exposure.

The cryptic species complex in the genus *Leptasterias* contains reproductively isolated species which may exhibit ecological differentiation with respect to abiotic stresses at specific locales but the present study has not addressed those interactions because of difficulties in segregating species. Three species which are reproductively isolated were found to contain only 1.5–3% first generation hybrids between the three species identified on San Juan Island, WA (Foltz, 1997; Foltz and Flowers, 2006). Their sampling at Mitchell Bay, WA indicated the species composition to be 24% *L. aequalis* A, 28% *L. aequalis* B, and 48% *L. hexactis* (Foltz and Flowers, 2006). The overwhelming majority of Leptasterias spp. at Sunshine Cove, AK are *L. alaskensis with L. hexactis* comprising several percent of each collection (Hrinovich et al, 2000).

The activity coefficient of *Leptasterias* spp. is an excellent early indicator of their longer term temperature tolerance limit as shown by their lack of ability to right at ambient temperatures above their longer term LC_{50}water temperatures. Within their zone of capacity adaptation to water temperature, sea star activity coefficients tend to be highest at intermediate water temperatures. Activity coefficient variation as a function of seasonal changes in ambient water temperature is not as sensitive as was observed for variation in activity coefficients as a function of ambient salinity (Shirley and Stickle, 1982a, b). Emersion for a time period typical for intertidal emersion is most stressful to *Leptasterias* spp. at temperatures experienced during warm summer days.

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