LOMA LINDA UNIVERSITY
The School of Science and Technology
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Responses to Salinity of Color Polymorphs in Two Populations of the Sea Star, *Pisaster ochraceus*

by

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A Thesis submitted in partial satisfaction
of the requirements for the degree of
Master of Science in Biology

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Each person whose signature appears below certifies that this dissertation in their opinion is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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TABLE OF CONTENTS

TABLES

FIGURES

ABSTRACT

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Pisaster ochraceus was analyzed to determine if varying salinities, animal color, or location affect the physiology of these sea stars. The three responses analyzed were aerobic respiration, ammonia excretion, and self-righting. The tested variables included two different color morphs (orange and purple) of *P. ochraceus,* two different locations (open coast and inland straits) in Washington State, and three salinities (22, 30, and 40 psu).

Wet mass-specific oxygen consumption rates were not significantly affected by color, location, or salinity, and Dry mass-specific oxygen consumption rates showed no significant differences for main effects, but a three-way interaction was identified. Similarly, ammonia excretion rates were unrelated to color, location, or salinity. Self-righting times were significantly different with color, location, and salinity.

Although measurements from the three experiments carried out in this study do not all point to differences in responses of the two color morphs, they nonetheless provide some evidence that color and location both have a significant effect on self-righting times at the three salinities tested. The results of my study suggest that, within a certain range (± 8 psu), *P. ochraceus* appears to be able to maintain normal aerobic respiration and ammonia excretion. However, when stressed to greater extremes outside of the range they are able to cope with, such as salinities of ± 10 psu or greater, their basic functions of mobility, such as self-righting responses, may be impaired.

CHAPTER ONE

INTRODUCTION

Natural History of *P. ochraceus*

The ochre sea star, *Pisaster ochraceus* (Brandt, 1835), is a benthic echinoderm in the class Asteriodea and is common and well distributed from Prince William Sound, Alaska, to Cedros Island, Baja California (Ricketts *et al.*, 1985). This species inhabits the lower limits of the intertidal zone as well the subtidal zones down to 97 meters, but is most abundant in the middle to lower intertidal zones that are exposed to waves or currents (Morris *et al.*, 1980; Lambert, 2000). *P. ochraceus* can usually be found on rocky substrates of the open coast. This predatory species is one of the most conspicuous and colorful of the intertidal fauna (Ricketts *et al.*, 1985). The species name, *ochraceus,* is derived from the Greek word *ochros,* in reference to the pale yellow or ochre color of some specimens (Lambert, 2000).

Pisaster ochraceus is commonly called the ochre or purple sea star, or the common starfish (Feder, 1957), and is not to be confused with the "common starfish" *Asterias rubens,* found on the Atlantic coast (Hendler *et al.*, 1995). Specimens of *P. ochraceus* generally range from 15 to 36 cm diameter (Ricketts *et al.*, 1985), and although smaller individuals are difficult to find, juveniles can be found in crevices and under rocks (L. McCloskey, *pers. comm.*). These organisms begin their lives as pale orange to salmon pink eggs 150 to 175 µm in diameter (Lambert, 2000). Around six days after the egg has been released, a planktotrophic (feed on other plankton) bipinnaria larva forms which lasts for up to two months. Following this stage, a bilaterally symmetrical brachiolaria larva develops, which attaches to the substrate and

metamorphoses into a radially symmetrical juvenile sea star (Lambert, 2000). Juveniles reach maturity in around five years and can live longer than 20 years as adults. A large adult individual can release approximately 40 million eggs in one spawning season (Millott, 1967; Brusca and Brusca, 2003).

Anatomy of Sea Stars

The general body plan of *P. ochraceus,* as in all other asteroids, is stellate (star shaped) and members of this species possess a pentamerous radial symmetry with body parts organized around an oral-aboral axis. Asteroids have a central disk and symmetrically projecting arms, which are called rays (Hendler *et al.*, 1995). Sea stars differ from other members of the phylum Echinodermata in that they have five or more open furrows, called ambulacral grooves, that are found on the underside, or oral surface, of their rays. These ambulacral grooves bear rows of tube feet, digestive glands, and gonads radiating into each ray. Members of *Pisaster ochraceus* usually have five stiff arms, a highly arched disc and a sunken mouth. The aboral surface contains a formation of white spines that form a net-like pattern. Papulae, also called gills or dermal branchea, lie between the spines. These papulae are thin-walled extensions of the coelom that protrude between skeletal plates and allow the exchange of respiratory gasses between the surrounding sea water and the organisms' internal fluid. They are thought to assist in the coloration differences of individuals, since this is where pigmentation occurs. Between the papulae are several types of pedicellariae, or pincher-like appendages, that may play a role in preventing microorganisms from settling on the skin of the sea star. These pedicellariae structures respond to external stimuli independently of the sea stars'

main nervous system and possess their own neuromuscular reflex components. The types of pedicellariae found in *P. ochraceus* are furcated, crossed, lanceolate and straight toothed, with the furcated form being most characteristic of this species (Millott, 1967; Lambert, 2000; Brusca and Brusca, 2003).

Sea stars are known for both their appetite as well as their diverse feeding strategies (Hendler *et al.*, 1995) and *P. ochraceus* are known to be voracious predators of low intertidal zone invertebrates (Ricketts *et al.*, 1985). They feed primarily in the summer and prefer mussels, barnacles, limpets and snails. However, at least 30 prey items have been documented and their diet depends on the availability of prey (Feder, 1959). One of the most common prey items of this organism is the California mussel, *Mytilus californianus,* which is abundant on wave-exposed, rocky substrates. *Pisaster ochraceus* eats mussels by inserting its stomach between the shells, secreting digestive enzymes and simultaneously pulling the bivalves shells apart with its tube feet (Morris *et al.*, 1980; Lambert, 2000).

The specialized tube feet of *P. ochraceus* are fleshy projections that contain external hollow suckers, called podia, that are used for locomotion, gas exchange, feeding, attachment and sensory reception. They are operated hydraulically by a unique coelomic water vascular system which contains a complex of fluid-filled canals with a single opening on the aboral surface known as the madreporite, or sieve plate. The water vascular system also aids in the internal transport of the coelomic fluid (Brusca and Brusca, 2003).

Ecological Importance of *Pisaster ochraceus*

Being a principal predator in intertidal areas of the Pacific coast (Johnson, 1976), *P. ochraceus* plays an important ecological role in intertidal community dynamics. Paine (1966) has shown in classical ecological studies that *P. ochraceus* is a keystone predator that has an amplified effect on the structure and diversity of the intertidal areas it inhabits. Paine (1966; 1969) and others (Menge and Sutherland, 1987; Garza, 2005) showed, that without sea star predation on *Mytilus californianus*, the rocky intertidal community shifted from a diverse mix of algae and invertebrates to a dominance of *M. californianus.* Sweere (unpublished) also studied the direct and indirect effects of *P. ochraceus* on tide pool communities and found that in the presence of *P. ochraceus*, there was a decline in the richness and diversity of other mobile tide pool species in experimental pools.

Although we understand much about the ecological role of this important species, much less research has been done on the causes of their most obvious character – their vivid color polymorphism. Understanding the significance and cause of this variation is almost completely lacking in the literature.

Color Polymorphism

Adult *Pisaster ochraceus* occur in a wide range of color morphs. Specimens are most commonly seen as purple, orange, brown, and less often in yellow, red, and pink (Ricketts *et al.*, 1985; Raimondi *et al.*, in press).

Vevers (1966) reviewed pigmentation in echinoderms and confirmed that they are among the most brightly colored of all marine animals and that nearly all their coloration is due to pigments. In Asteroids, the most widespread chemical pigments are the

carotenoids. Carotenoids are yellow to red pigments of aliphatic or alicyclic structure, composed of isoprene units (Karrer and Jucker, 1950). These integumentary carotenoids are often linked to proteins to form water-soluble carotenoproteins. In asteroids, the principal carotenoids found are β-carotene, cryptoxanthin, echinenone, astaxanthin, and one or more keto-carotenoids, as well as traces of xanthophylls. Because carotenoids are essentially plant pigments which animals are thought to be incapable of synthesizing *de novo* (Karrer and Jucker, 1950; Fox and Hopkins, 1966; Vevers, 1966), it seems likely that the coloration of *P. ochraceus* is at least partially controlled by diet.

Studies by Fox and Scheer (1941) shed light on the fact that *Pisaster ochraceus* stores considerable amounts of mytiloxanthin, a carotenoid found specifically in its main prey item, *Mytilus californianus* (which gains these pigments through its diet). They hypothesized that a carnivorous organism such as *P. ochraceus* would obtain more oxygenated carotenoids through its diet than would an herbivorous organism. To my knowledge, no published studies have tested the genetic basis for incorporating plant pigments, such as carotenoids, into the integuments of animals. How environmental factors play a role in the survivability of different color morphs is not known

Humphreys (2003) observed that in protected waters like those in the Pacific Northwest, there are more purple individuals, whereas on exposed coasts, the seastars tend to be more orange or brown. It is unknown whether these polymorphs are really genetically fixed morphs, or rather are color phases that may change during the course of the organism's growth and development. Rearing studies have not been carried out which could shed light on how environmental factors such as salinity, diet, temperature, pH, and

UV exposure play a role in the ontonogenic determination of color (C. Harley, *Pers. Comm*).

Raimondi et al. (2004; *in press*) reviewed common explanations for color polymorphism in animals and applied them to *P. ochraceus.* They determined that non-random mating, apostatic selection (which is a negative frequency-dependant selection pressure based on predation pressures), and disruption through crypsis, would not support the basis for polymorphism in *P. ochraceus*. They did, however, identify a consistency in frequencies of orange to purple color morphs. They sampled 26 sites along the open coast from Southern California to Northern Oregon, and found consistently that the range in percent orange morphs for all sites was 12.6 % - 27 % (mean = 20.0 % ± 4.4 % s.d). This consistent frequency of percent orange, however, is not exhibited throughout the protected waters in the Inland Straits[1] in Washington State. Individuals found in the Inland Straits tend to be larger than the coast populations and contain a higher frequency of purple, lower frequency of orange, and no brown individuals (Raimondi et al, *in press*, D. Cowles, *pers. com.*).

Harley et al. are presently comparing the genetic structure of Inland Straight and open coast populations in British Columbia, Canada (C. Harley *pers. com.*). To date, they have found no major genetic differences between these populations. This seems unsupportive of the more obvious possibilities of a founder effect or incipient speciation between the populations that may be assumed under these circumstances (Bilton *et al.*, 2002).

[1] Refers to protected bodies of marine waters found inland in North-Western Washington State including the Strait of Juan de Fuca, the Puget Sound, and the waters adjacent to the San Juan Islands.

These data suggest that another possibility is for certain color morphs to have different physiological tolerances to environmental factors. If, for example, orange individuals were more sensitive than purple individuals to certain ecological factors present in the inland straits, a prediction about the distribution of colors between the two locations could be tested.

Environmental Variations Between the Open Coast and Inland Straits of Washington State

A look at the spatial distribution of color morphs of these organisms may provide evidence that the cause of color polymorphism in *P. ochraceus* is directly related to its environment. Sea stars, such as *P. ochraceus* that inhabit the intertidal zone are exposed to great fluctuations in water temperature and, to a greater extent, air temperature in their natural environment. While the coastal waters of Washington State usually do not drop below 10˚C or rise above 16˚C, Feder (1957) has shown that *P. ochraceus* will tolerate air temperatures of up to 21˚C in the laboratory for at least three hours. This may be partially due to the fact that these organisms are able to keep their own temperatures down to some extent by evaporative cooling.

Studies of other asteroids have shown that temperature has an effect on rates of locomotion as well as self-righting behavior, which is a measure of a turnover response time after placing the animal on its aboral surface (Feder and Christensen, 1966).

Kinne (1963) points out that organisms tend to adapt to their total environment rather than to isolated factors. One of the most important physical factors that can exert pressure on the survival of marine organisms, along with temperature, is salinity. An

example of how this interaction has an effect on sea stars was demonstrated on *Solaster endeca* by Ursin (reviewed in Feder and Christensen, 1966). This seastar avoids areas with a mean temperature >14˚C in the warmest month, but also requires a salinity of at least 30 practical salinity units (psu). Schlieper (1956, in Feder and Christensen, 1966) and Kowalski (1955, in Feder and Christensen, 1966) studied the asteroid, *Asterias rubens,* from two distinct environmental salinities (30 psu and 15 psu). Data from these experiments showed that lower salinity populations took longer to right themselves when exposed to higher temperatures. They suggested that this decreased response is related to the higher content of water in the tissues of the organisms found in the more hypo-saline environment. Brattstrom (1941, in Feder and Christensen, 1966) and Ursin (1960, in Feder and Christensen, 1966), have documented the distribution of sea stars as a function of salinity.

Lowered tolerance for hypo-saline conditions is well documented in some asteroids. Smith (1940; in Feder and Christensen, 1966) found that *Asterias vulgaris* could survive in the laboratory for at least three days at a salinity of 14 psu at 20˚C. Field sampling showed, however, that that no individuals were found in environments containing salinities below 23 psu.

The environments between the open coast and the Inland Straits of Washington State are very distinct, with the open coast having a higher salinitiy (with an average of 35 psu), warmer water temperatures, more cloudy and foggy days, and consistent wave exposure (D. Cowles *pers. comm.*). In contrast, inland waters have lower salinities with an average annual salinity of 29 psu (NEER, 2006), consistently cooler water temperatures and limited wave exposure. The hypo-saline conditions in the protected

waters of the Pacific Northwest are due to the size of the Inland Straits' watershed and surface runoff from over 10,000 rivers and streams (Finlayson, 2004).

The bodies of water associated with the Inland Straits are considered estuarian. An estuary can be defined as an inlet where sea water is diluted by the inflow of freshwater, or simply as a river with variable salinity due to the mixing of seawater (Green, 1968). The Rosario Strait in which the Inland Straits populations were collected for the current study was adjacent to the Strait of Juan de Fuca.

The Strait of Juan de Fuca, Georgia Straits, and Rosario Strait, are tidally dominated, but have estuarine components (Holbrook and Halpern, 1982). Southerly winds push water against Vancouver Island, which forms the northern portion of the mouth of the Strait of Juan de Fuca. These winds reverse the sea-surface slope in the Strait, resulting in a landward intrusion of fresh, warm surface water and seaward retreat of deep, more saline water (Cannon, 2003).

In contrast to the dynamic mixing of the inland waters, the Olympic coastal areas of Washington State maintain temperatures and salinities which are kept more constant throughout the year. Factors that contribute to this consistency include shallow continental shelves that prevent coastal upwellings and consistent fog-induced temperature regimes (Sanford, 1999; 2001). The open coast is said to be remarkably stable in its physico-chemical conditions (Morris *et al.*, 1980) in comparison to the inland straits.

Salinity Tolerance in *P. ochraceus*

My research examined the effects of salinity on three color morphs of *P. ochraceus* from two environmentally distinct populations of the open coast and the inland straits of Washington State. Salinity tolerance was chosen due to the fact that echinoderms are reported to have poor abilities to osmo- and iono-regulate, and lack evidence of excretory systems (Stickle and Diehl, 1987; Brusca and Brusca, 2003). The choice to test acute responses to salinity was also made due to a review of the literature which revealed studies identifying biochemical, reproductive, developmental and morphological responses to reduced salinities in other echinoderms. Acute responses to salinity stress can be manifested as swelling and stiffness due to turgor pressure, increase in weight due to increased water content, and loss of integumentary pigmentation resulting in a blotchy appearance due to damaged epidermal tissue (Binyon, 1972a; 1972b; Stickle and Diehl, 1987).

Roller and Stickle (1985) state that postmetamorphic *Pisaster ochraceus* are not found in brackish water (hyposaline conditions). They have shown through experiments, that larvae will survive salinities of 20 psu for only 32 days. This represents little more than 10% of their planktonic existence that can last 228 days at salinities of 30 psu, which is closer to their normal environmental salinity.

Echinoderms are osmoconformers, and since their coelomic fluid usually remains isoosmotic to the environment (Binyon, 1966), I tested the hypothesis that acute changes in salinity differentially affect responses, such as metabolic rate, ammonia excretion, and self righting, of the two color morphs. Studies of this kind have been done in other echinoderms (Sabourin and Stickle, 1981; Shirley and Stickle, 1982; Forcucci and

Lawrence, 1986; Talbot and Lawrence, 2002) as well as in other marine invertebrate species (Aarset and Aunaas, 1990; Jury *et al.*, 1994; Dunbar and Coates, 2004).

Rankin and Davenport (1981), discuss the problems marine intertidal organisms face in maintaining their internal salt concentration. All echinoderms are either euryhaline or stenohaline osmoconformers. Osmoconformers respond to reduced salinity by absorbing water and excreting salt until their bodies are isosmotic with the external medium. The problem for osmoconformers like *P. ochraceus* is that they gain a large amount of water before reaching osmotic equilibrium causing an increase in weight and a swelling of body size, which can impair body activities such as locomotion and food collection (Green, 1968).

Pisaster ochraceus is a stenohaline osmoconformer so it cannot eliminate the excess fluid gained by osmosis by producing dilute urine, but must rather remain swollen in hypo-osmotic salinities. However, Binyon (1972b) suggests that echinoderms are not as stenohaline as they were once considered to be as they can be found from salinities from 8 psu to 46 psu. Regardless, salinity has been shown to affect echinoderm physiology, feeding rates, growth, and metabolism (Stickle and Diehl, 1987). Additionally, lower salinities have been shown to reduce the spawning intensity of *Asterias rubens* (Thorsen, 1946, in Binyon, 1972b)

Salinity is also a potential factor influencing color polymorphism. Studies with the crinoid, *Tropiometra carinata,* showed a reduction of pigmentation from the integument in brackish waters with salinities as low as 12 psu (Clark, 1917, in Binyon, 1972b).

Oxygen Consumption and Aerobic Metabolism in *P. ochraceus*

Newell (1979) reviewed factors affecting the rate of oxygen uptake in intertidal organisms. The amount of oxygen available to intertidal organisms is determined by the surrounding sea water, which contains a maximum concentration of 5 to 8.5 ml O_2 $liter^{-1}$. The lower solubility of oxygen in sea water means that intertidal organisms must develop relatively large respiratory surfaces through which gas exchange occurs. Some intertidal organisms utilize respiratory pigments to facilitate this gas exchange, however, studies have not linked pigmentation, nor the color polymorphism found in *P. ochraceus,* to this respiratory function.

Body size and activity resulting in energy expenditure can complicate testing the effects of environmental factors in marine intertidal organisms. The effects of salinity on oxygen consumption can also be confounded by other external environmental variables, such as temperature and external oxygen availability. Newell (1979) stated that an increase in respiration rate, which has commonly been observed under conditions of salinity stress, may thus mainly reflect increased activity rather than the energetic cost of osmoregulation. Another problem with measuring the effect of salinity on echinoderm metabolism is that a small part of the metabolic rate may be due to anaerobic processes (Shick, 1983). However, the analysis of anaerobic respiration is beyond the scope of this study.

Project Goals, Objectives and Significance

The goal of this project was to clarify how abrupt changes in environmental salinity influence physiological responses of different color morphs of marine intertidal invertebrates. Acute responses to salinity were measured using three different methods: aerobic respiration (n=72), ammonia excretion (n=34) and self-righting (n=36).

Two different color morphs (orange and purple) in *P. ochraceus* from two different locations (open coast and inland straits), at three experimental salinities (22, 30, and 40 psu) were tested to determine acute physiological and behavioral responses. I tested the hypothesis that acute changes in salinity differentially affect responses, such as metabolic rate, ammonia excretion, and self righting, of the two color morphs. Due to the role of *P. ochraceus* as a keystone predator, a small shift in response or color frequency could have amplified effects on the intertidal ecology and structure of some inshore communities.

CHAPTER TWO

MATERIALS AND METHODS

Collection Methods

Adult *Pisaster ochraceus* were collected from rocky intertidal zones at two sites at each of two locations (see Figure 2.1 and Table 2.1). One of the collection locations was on the open coast of Olympic National Park (ONP), wheras the other was in the inland waters of Rosario Straits. Open coast collections were made under a National Park Service (NPS) Permit (# OLYM – 2006 – SCI – 0149), and Inland Straits collections were made under a Washington Department of Fish and Wildlife Permit (# 06-203).

Seventy-two specimens were collected between the two sites and transported to the laboratory of the Walla Walla College Marine Station (WWC-MS; N48˚24'58.13", W122˚39'04.81"). Of the 18 specimens collected at each site, nine were orange, and nine were purple. This dichotomy of color followed the methods described in Raimondi *et al.,*(*in press*), with all darker individuals (purples and browns) classified as purple. The 36 specimens from the open coast were transported in 18.9 L plastic buckets containing seawater which were placed on ice in larger bins, thereby maintaing cooler temperatures during transport to the WWC-MS. The 36 specimens from the Inland Straits were collected by boat from rocky intertidal areas near the WWC-MS, making temperature control unnecessary during transport. These specimens were also transported in 18.9 L plastic buckets with seawater.

Only individuals free of any external evidence of disease, epibionts, or arm regeneration were collected. Individuals of ambiguous or intermediate coloration were excluded.

Specimen Maintenance

Sea stars brought back to the laboratory were maintained in running sea water in an average salinity of 30 psu at 12˚ ± 1˚C. Artificial lights were kept on during the day and turned off from 23:00 to 08:00 hrs. Specimens from different sites were kept in separate sea tables and no more than 18 individuals were kept together in each holding tank. Individuals from all collection sites were kept under the same regimes of feeding, artificial light, temperature, and salinity. Specimens were maintained in aquaria for 7 d with an initial feeding and subsequent starvation to allow for acclimation to take place. Individuals were not tagged due to their rigidity and ability to shed tags (C. Robles, *pers. com.*).

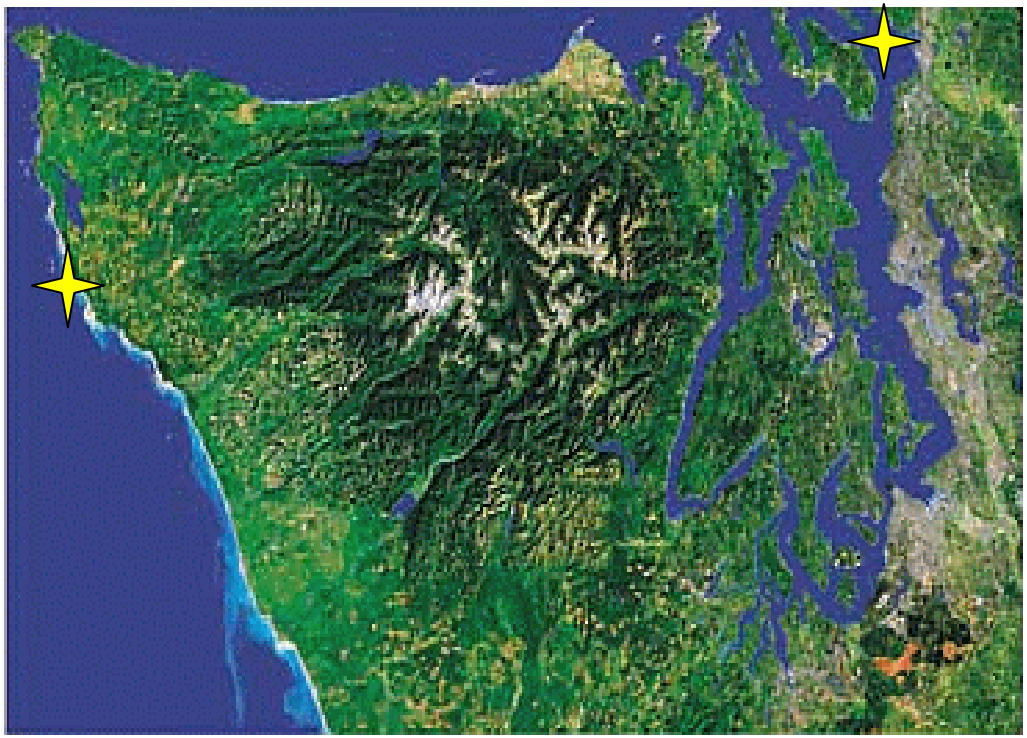

Figure 2.1. Northwestern Washington state showing the two different locations (✦) where *Pisaster ochraceus* were collected. Adapted from http://www.adsat.com/thumnail/catalog/olympic.htm

Table 2.1. Spatial, temporal, and salinity data for the four collection sites within the two locations

Location	Date	Collection Site	Time	Geographic Coordinates	Environmental Salinity
Open Coast	6/25/2006	Hole in the wall – ONP	6:45-8:45	N47˚56.505' W124˚39.120'	35 psu
Open Coast	7/12/2006	Beach 4 – ONP	16:00-18:00	N47˚39.08' W124˚23.31'	35 psu
Inland Straits	7/9/2006	Huckleberry Island - InSt	8:00-9:00	N48˚32.137' W122˚33.997'	29 psu
Inland Straits	7/26/2006	Burrows/Guemes Is - InSt	11:00-14:00	N48˚28.428' W122˚41.139'	29 psu

Aerobic Respiration

A flowchart of the experimental design of the aerobic respiration study is given in Figure 2.2. Of the 72 organisms used in this experiment, 36 specimens were from the open coast and 36 were from the inland straits. For each of the two groups of 36, 18 individuals were purple and 18 were orange. All individuals were tested in one of three salinities: 22, 30, or 38 psu. A salinity of 30 psu was chosen as the control because this was the acclimation salinity for all organisms. Thirty-eight psu was chosen as the hypersaline condition, and is higher than any salinity this organism is normally found in. Twenty-two psu was chosen as the hyposaline condition, and is lower than any salinity that that this organism has been found to live in.

Four metabolic chambers (A, B, C, and D) were used. Specifics for each chamber are listed in Table 2.2 and photos of these chambers can be seen in Figures 2.3, 2.4, and 2.5.

Metabolic chambers A and B were constructed of 17.0 L plastic buckets. Lids were constructed from a 1.27 cm thick sheet of clear acrylic cut to fit the chambers. The lid and chamber were separated by a rubber gasket attached to the chamber using aquarium silicone. Holes were drilled into these lids for the dissolved oxygen probe (which was mounted in a rubber stopper), the intake valve, the exhaust valve, and the power chord for the submersible pump. These holes were also sealed using aquarium silicone. Rio® Mini 50 Aqua Pump/ Powerheads were used to circulate water in the chambers. These two chambers were placed directly on seawater tables which acted as water baths to maintain a constant temperature of 12˚ ± 1˚C.

Chambers C and D were designed by Dr. David Cowles (Walla Walla College), and were water-jacketed models with temperature maintained by a VWR™ 1160S water chiller. Water in these two chambers was circulated by a magnetic stir bar with both chambers sitting on magnetic stir plates.

The probe for chamber C was mounted directly to the chamber lid with chamber water directed past the probe by a current that was generated by a magnetic stir bar. Chamber D had a magnetic stir bar as well as additional circulation generated by a Manostat™ E-Series Peristaltic Pump. This device pumped water into a separate flow-through chamber (Figure 2.6) which housed the probe. The probe housing had its own stir bar and magnetic stir plate to ensure adequate flow past the probe.

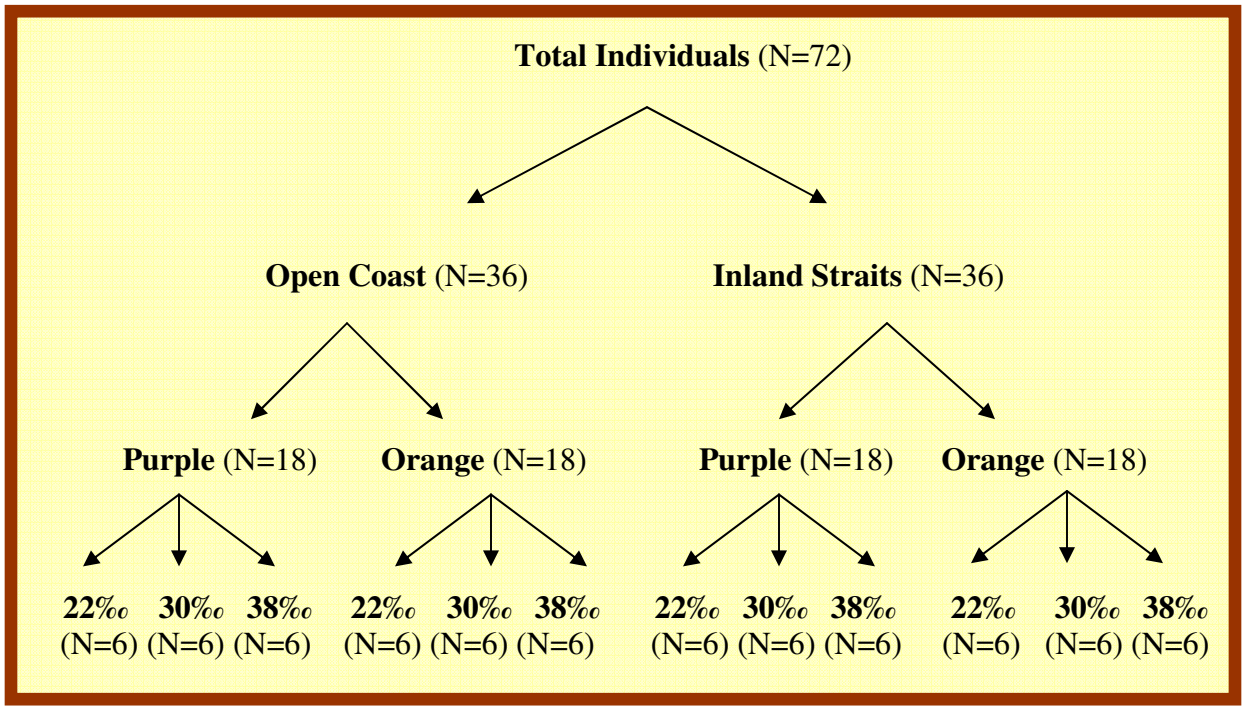

Figure 2.2. Flow-chart displaying the experimental design of the aerobic respiration study.

Table 2.2. Details for each of the four metabolic chambers (A-D) used in the aerobic respiration experiments. Volumes for chambers A and B are reported for both a full chamber volume as well as a chamber volume that was reduced by six plastic bottles placed in the chamber with smaller sea stars.

Chamber	Volume (ml)	Dissolved O_2 Probe	Meter	Software Package
A	17230/15950	YSI 5739	TPS 90-D	Win-TPS
B	17230/15950	YSI 5739	TPS 90-D	Win-TPS
C	5680	Nester DO Probe	Nester Instrument Analogue Meter	Probes
D	6025	Hach Sens-Ion DO Electrode	Hach Sens-Ion 8	Hach Link 2000

Figure 2.3. Chambers A and B with experiment in progress. Chamber A is in the foreground and chamber B is in the background.

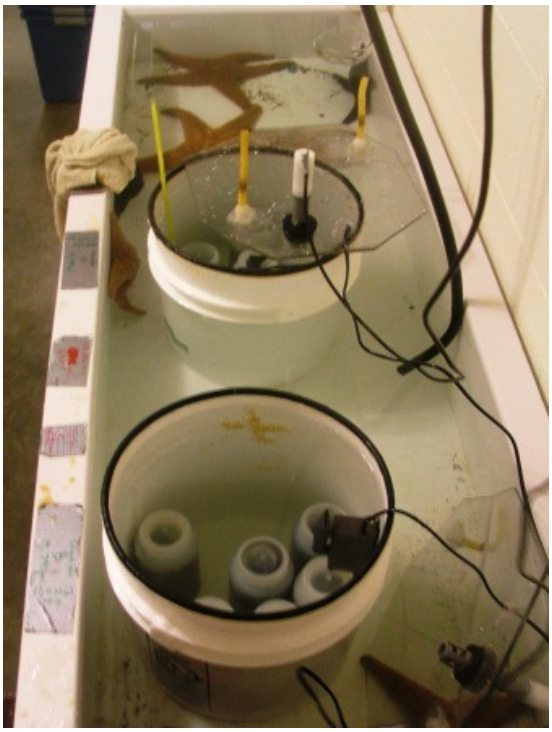

Figure 2.4. Chambers A and B empty and with lids off. Notice the submersible pumps, volume reduction bottles, and rubber gaskets. Chamber B is in the foreground.

Figure 2.5. Chambers C (right) and D (left). The housing unit for the chamber D probe is between the chambers and the water chiller is the unit on the right. Note the three magnetic stir plates; one plate is under each chamber and one is under the probe housing.

Figure 2.6. The probe housing for chamber D on top of a magnetic stir plate. Note the hoses going in and out of the housing; one goes out to the chamber and one is coming in from the peristaltic pump.

Water for the aerobic respiration experiment was prepared by pouring sea water obtained directly from the shore at the WWC-MS through a mesh-screen filter to remove macro-algae particles. Salinity was diluted using either commercially distilled water or deionized reverse-osmosis water. Instant Ocean™ sea salts were used to increase salinity. The volumes of chambers A and B were reduced for measuring metabolic rates of smaller individuals by using six plastic bottles (640 ml each) to displace water volume in the chambers (Fig. 2.4). This allowed the probes to be more sensitive to changes in dissolved oxygen concentrations for smaller specimens. Larger individuals were run in the chambers without chamber volume reduction.

Once water was in the chambers, it was aerated by using 60 cc syringes to blast (squirt) water repeatedly into the chambers. Chambers A and B were blasted 20 times and chambers C and D were blasted 15 times (since they had less volume) to ensure adequate saturation of oxygen. Figure 2.7 shows a metabolic chamber being aerated by this method.

Once chambers were air saturated, an organism was selected from the acclimation tables. Sea stars were measured for oral length radius, from the tip of the longest ray to the mouth at the center of the oral disk. A wet mass was measured by first dabbing the specimen with dry paper towel until pooled water on both aboral and oral surfaces was absorbed and the animal was then weighed on a balance scale (± 1.8 grams) (see Figure 2.8).

Organisms were placed in chambers and lids sealed with eight spring clamps per lid for chambers A and B, or eight wing-nut screws on chambers C and D. Dow Corning™ High Vacuum Grease - Silicone Lubricant was applied to the rubber gaskets

on all four chambers to ensure a proper seal. Silicone stopcock grease is an inert substance routinely used in metabolic work to seal the containers (D. Cowles, *pers. comm.*). Air bubbles were removed from chambers by adding water with a syringe through the intake valve while the chamber was tipped slightly to remove excess air bubbles from the chamber through the exhaust valve. The dissolved oxygen probes were then calibrated in the saturated water to 100 % and data were logged every 60 sec.

Metabolic rates were obtained by analyzing the slopes of oxygen saturation vs. time over the range of 95 - 80 % oxygen saturation, allowing the animals and probes to stabilize before data collection began. Ending at 80 % minimized the possibility of oxygen-dependent respiration. Chamber temperature was recorded and a displacement volume for each organism was obtained by placing the organism in an apparatus that consisted of a plastic bucket and a spout to direct the displaced water. Displacement volume was measured by recording the amount of water that overflowed into a graduated cylinder when the animal was placed in the apparatus (Figure 2.9). The displacement volume was used to calculate the effective volume of the metabolic chambers by subtracting displacement volume from the total chamber volume.

Figure 2.7. Chamber D being aerated with a 60 cc syringe.

Figure 2.8. A purple specimen being weighed on a balance scale before being placed in a chamber.

Figure 2.9. The displacement apparatus with a graduated cylinder being filled with water displaced by a sea star.

Once aerobic respiration data were obtained, outliers, identified by regression as standardized residuals ≥ 2 or ≤ -2, were removed. Data were then smoothed by taking a running average of the five data points adjacent to each point plotted on a graph. The slope of the best-fit linear trend line to the cleaned and smoothed data was used in obtaining the metabolic rate. An example of a plotted slope can be seen in Figure 2.10.

To report metabolic rates per gram of dry tissue, 36 of the 72 individuals used in this study were placed in a drying oven (Lane Science Equipment) at 60˚C for at least 72 hrs. Dry masses were then obtained on an electronic scale (Mettler Toledo PL202-S) and a regression slope was calculated to interpolate dry masses for sea stars that were not dried. A preliminary analysis showed that even large sea stars were dried to constant weight by this procedure.

Once oxygen consumption slopes and interpolated dry masses were obtained for all individuals, air saturation values were calculated as milligrams of oxygen for each of the experimental salinities and temperatures. These values were from The Oxygen Solubility Tables for water exposed to water-saturated air at 760 mm Hg pressure (YSI-Incorporated, 2006). Metabolic rates were calculated using the formula:

$$M_R = R_C \bullet S_A \bullet V_C \bullet M^{-1} \bullet T^{-1} \quad (1)$$

where M_R = metabolic rate, R_C = rate of O_2 consumption (slope), S_A = air saturation value (mg O_2), V_C = effective volume of chamber in liters, M = organism wet mass in grams, and T = time in hours. The metabolic rates were calculated and recorded as mass-specific rates per gram of wet tissue as well as per gram of dry tissue because the wet

masses of all organisms were not always directly proportional to their dry masses (R^2 = 0.887). Metabolic rates were reported in mg O_2 g^{-1} h^{-1} (wet or dry).

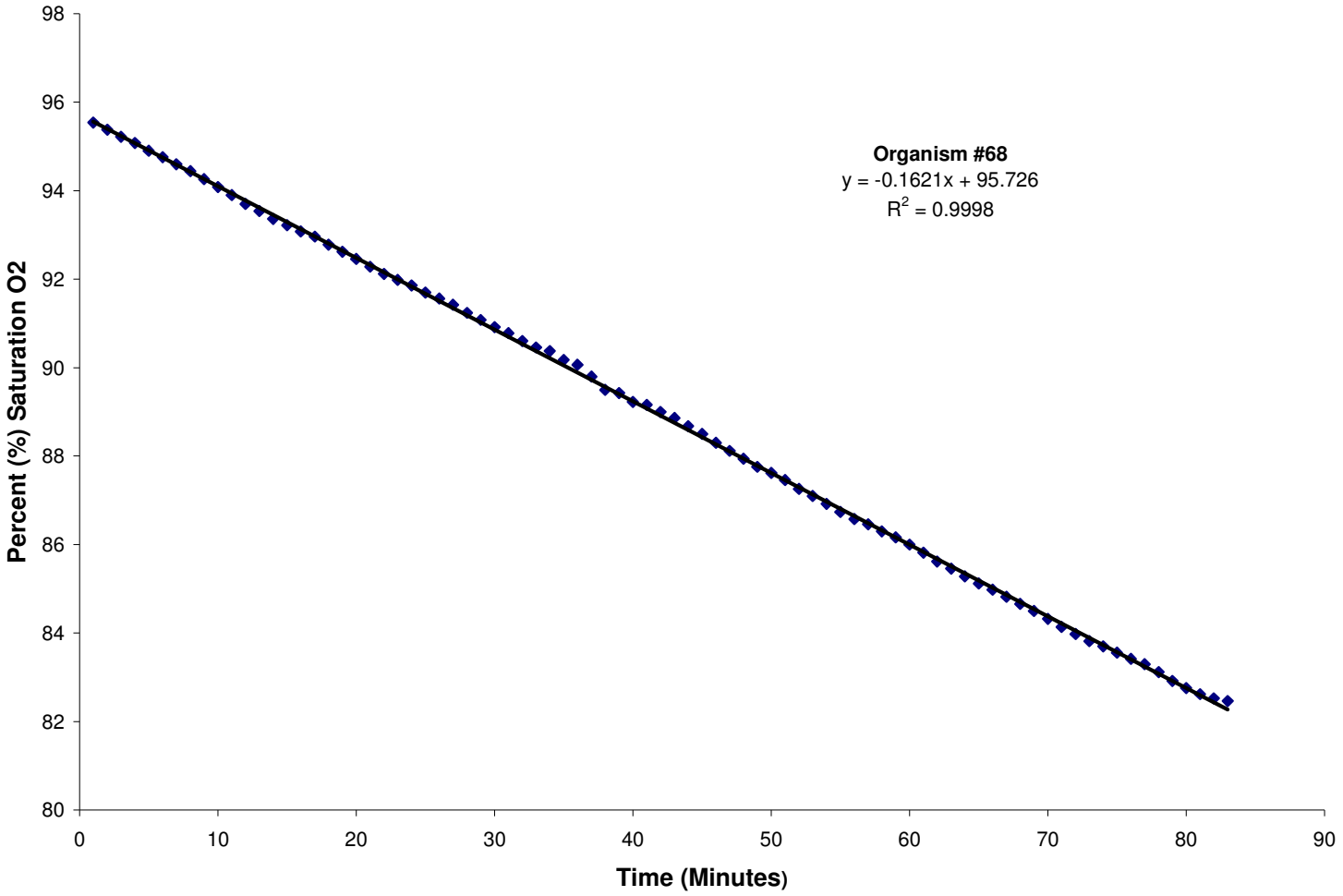

Figure 2.10. Sample of cleaned and smoothed data-chart plotting a final slope with standardized residuals ≥ 2 or ≤ -2 removed. The regression equation and r^2 value are provided.

Ammonia Excretion

Rates of ammonia excretion were obtained by extracting a 5 ml sample of chamber water using a micro-pipette at the start and end of 36 of the aerobic metabolism chamber runs. Eighteen of these 36 were from each of the two locations, and of each location, nine were orange and nine were purple. Of the nine individuals of each color, three were tested in each of three salinities (22, 30, and 38 psu). Two samples were spilled during vortexing, so a sample size of 34 was available for analysis. Pre-samples were collected immediately before introducing organisms into the metabolic chambers and post-samples were collected immediately after the chamber lid was opened. Start and stop times for the sealed chambers were recorded at the time of sample collections. The samples were kept in test tubes, covered with parafilm, and stored in a freezer.

Samples from the open coast were analyzed on July 7 and 8, 2006. These samples were refrozen for an additional 24 hrs. This was the only difference in methods between organisms sampled from the open coast and those sampled from the Inland Straits which were analyzed on August 8, 2006. Samples from this second subset were all analyzed within 24 hours of extraction from the metabolic chambers.

A salicylate-based Freshwater/Saltwater Ammonia Test Kit (Aquarium Pharmaceuticals, Inc.), containing two separate reagents, was used to analyze all 34 of the ammonia samples. Before analysis, samples were vortexed (VWR Standard Mini Vortexer) for 10 sec at a speed of 6. Three-hundred µL of reagent #1 was added and samples were vortexed again for 10 sec at a setting of 6. Three-hundred µl of Reagent #2 was then added to each sample followed by 20 sec of vortexing again at a setting of 6 and a 20 min incubation period at room temperature.

Standards were made using known amounts of ammonium chloride (NH_4Cl) and distilled water that had been mixed with enough Instant Ocean® to make the salinity 30 psu. Known concentrations of ammonia (NH_3) were made up in test tubes to a volume of 5000 µl. Standards were prepared for analysis using the same protocol as the start and end ammonia samples from the aerobic respiration experiments. The standard curve for the first 18 individuals tested was made using samples with 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 mg NH_3/L. The standard curve for the next 18 individuals was made using samples with 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg NH_3/L. These concentrations of NH_3 were used for the second standard curve because they were closer to the range of the physiological ammonia production under the current test conditions.

All samples and standards were filtered through 45 µm syringe filters and placed in standard cuvettes, then tested for absorbance at 690 nm (which is the range in which these samples peaked) using a Beckman DU 520 spectrophotometer and 1 cm pathway square cuvettes. Ammonia concentration in each experimental water sample was calculated by regression from the standard curve.

Ammonia excretion rates were obtained by using the formula:

$$E_A = C_E - C_S \bullet E_V^{-1} \bullet M^{-1} \bullet T^{-1} \bullet 1000 \qquad (2)$$

where E_A = ammonia excretion rate, C_E = ending ammonia concentration (mg NH_3), C_S = starting ammonia concentration (mg NH_3), E_V = effective volume in liters, M = organism wet mass in grams, and T = time in hours. The rates of ammonia excretion that were analyzed in this study were reported in µg NH_3 L^{-1} g^{-1} h^{-1}. Values that were too minute to

be detected by these methods resulted in negative values, which were converted to values of zero.

Self-Righting Experiment

Self-righting data were collected from 36 individuals. Three large, open-topped plastic chambers contained seawater salinities of 20, 30, and 40 psu, respectively. Chamber water was prepared using sea water from the shore of the WWC-MS. Water in the self-righting chambers was either diluted with deionized, reverse-osmosis water or concentrated with Instant Ocean® Sea Salts and salinity was measured using a hand-held refractometer.

Self-righting chambers were maintained at a constant 12.5 ˚C. Two organisms (one purple and one orange) were run simultaneously during each round in a chamber (Figure 2.11). Self-righting times were obtained by turning a specimen over on its aboral surface and using a stopwatch to measure the time until a portion of the oral surface of all five rays touched the bottom of the self-righting chamber and the mouth could no longer be seen from above. If individuals took longer than 1 hr to turn over, the timing was stopped and a value of 60 min was given for that individual. Subsequent to self-righting, the sea star was removed and the oral disc was measured from the tip of the longest ray to the center of the mouth.

Figure 2.11. Sea stars run simultaneously in a self-righting chamber. The top one is orange and bottom one is purple.

Figure 2.12. Tests of open coast individuals set up on the shore of the WWC-MS. The three self-righting chambers are in the water to the left.

Figure 2.13. Self-righting tests of Inland Straits individuals set up in an outdoor sea tank at the WWC-MS.

Statistical Methods

Both wet and dry mass-specific oxygen consumption rates were analyzed by a 2×2×3 (color × location × salinity) Analysis of Covariance (ANCOVA) model. Because there was a negative relationship between sea star body size (wet mass) and rank-transformed mass-specific oxygen consumption (wet-mass: $MR_{wet} = 57.570 – 0.056 \times$ size, $F_{1,71} = 22.870$, $p < 0.0001$, $r^2 = 0.246$; dry mass: $MR_{dry} = 43.711 – 0.019 \times$ size, $F_{1,71} = 2.078$, $p > 0.05$, $r^2 = 0.029$), body size was used as a covariate. Color and location were treated as between-subjects factors, salinity was treated as a within-subjects factor, and rank-transformed wet mass was used as a covariate. The data for wet and dry metabolic rates were rank transformed to meet assumptions of normality and homoscedasticity.

Rank-transformed ammonia excretion data were analyzed using a similar three-way Analysis of Variance (ANOVA) for color, location, and salinity.

Rank-transformed self-righting times were also analyzed by a three-way ANCOVA, with size used as a covariate and post-hoc Tukey tests applied to times for different salinities.

Data were analyzed by SPSS v13 (Statistical Package for the Social Sciences, Chicago, Illinois, USA) with an alpha level of $p < 0.05$.

I computed effect sizes as partial η^2 values, indicating the approximate proportion of variance in the dependent variable explained by each independent variable or interaction. However, partial η^2 values are frequently inflated (Cohen, 1988). Thus, when the partial η^2 values summed to >1 for a given model, I divided each value by the sum of partial η^2 values to obtain adjusted values.

CHAPTER THREE

RESULTS

Aerobic Respiration

The means of both wet and dry mass-specific oxygen consumption rates for the three factors analyzed are presented in Table 3.1 and the means for dry mass-specific rates are presented in Figure 3.1. Results of the ANCOVA showed no significant differences among color, location, or salinity for both wet and dry mass-specific oxygen consumption rates (main effects, $p > 0.05$; Table 3.2 and 3.3, respectively). However, there was a significant three-way interaction ($F_{2,59} = 3.866$, $P < 0.05$) between color, location, and salinity with regard to the dry mass-specific rates of oxygen consumption.

The orange sea stars from the both locations and the purple sea stars from the Inland Straits had the highest metabolism at 30 psu, whereas purple sea stars from the open coast had the highest metabolism at 38 psu.

Four separate two-way ANCOVA's were performed to determine the presence of a simple main effect. No significant effects were identified in all four combinations of tests. The means of dry mass-specific rates can be seen in Figure 3.1.

Though non-significant, inspection of these data presents trends that show Inland Strait populations overall (both orange and purple) have their highest mean metabolic rates when tested at their environmental salinity (30 psu).

Table 3.1. A comparison of means (± 1 S.E.) among groups for both wet and dry mass-specific oxygen consumption rates (mg O_2 g^{-1} h^{-1}), in *P. ochraceus* tested in 12 combinations of color, location and salinity (n = 72).

Color	Location	Salinity (psu)	N	Mean ± 1 S. E. (Wet) (mg O_2 g^{-1} h^{-1})	Mean ± 1 S. E. (Dry) (mg O_2 g^{-1} h^{-1})
Orange	Open Coast	22	6	2.55 ± .336	8.31 ± 1.07
		30	6	2.70 ± .761	9.80 ± 2.35
		38	6	2.37 ± .212	7.90 ±.473
	Inland Straits	22	6	1.59 ± .268	6.57 ± .964
		30	6	1.89 ± .247	7.61 ± .564
		38	6	1.80 ± .104	7.51 ± .383
Purple	Open Coast	22	6	1.70 ± .226	6.67 ± .973
		30	6	2.42 ± .453	8.79 ± 1.330
		38	6	2.49 ± .160	9.76 ± .573
	Inland Straits	22	6	1.61 ± .236	7.29 ± 1.102
		30	6	1.88 ± .146	8.27 ± .652
		38	6	1.47 ± .094	6.43 ± .325

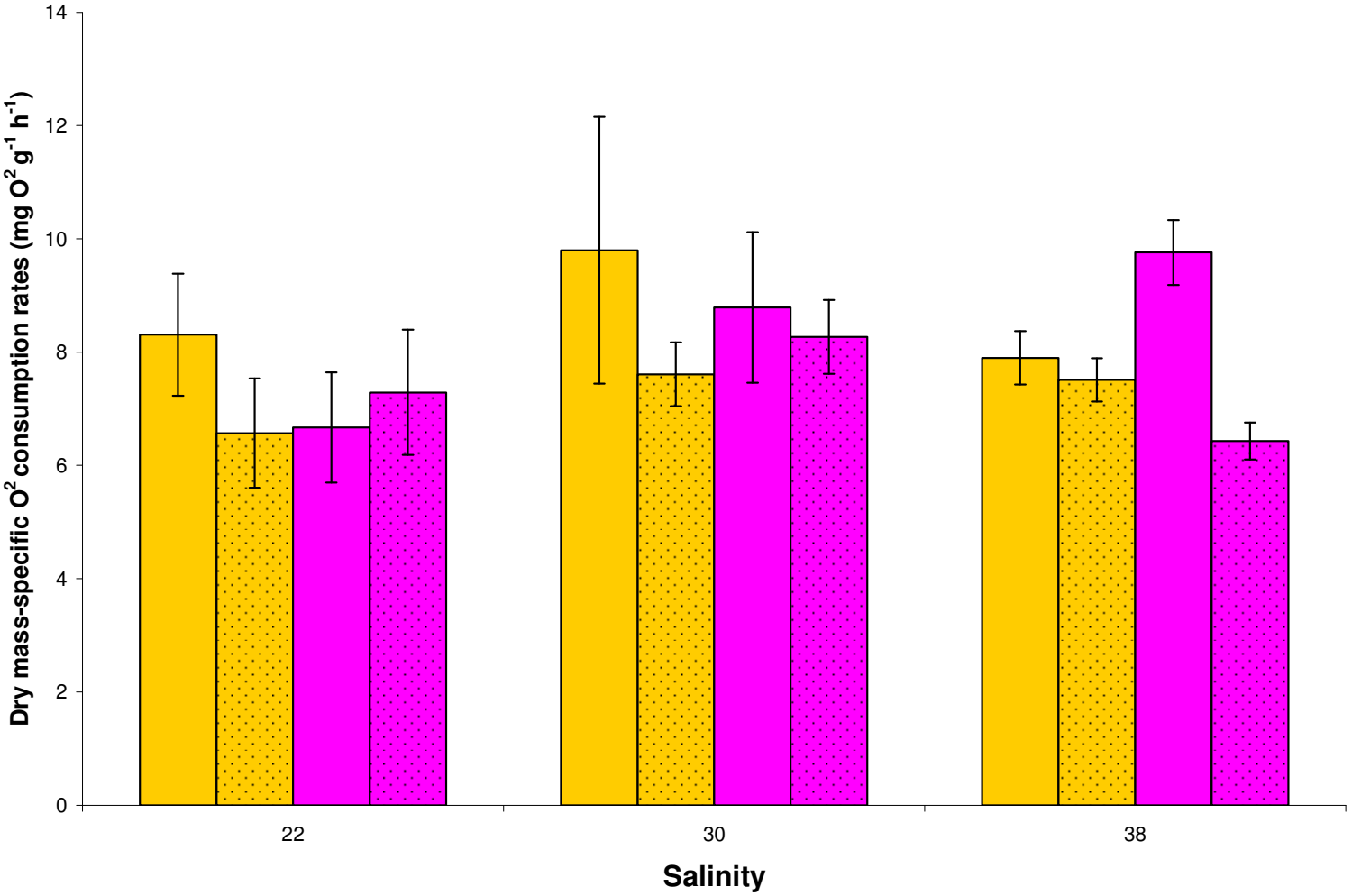

Figure 3.1. A comparison of means (± 1 S.E.) among groups for dry mass-specific oxygen consumption rates (mg O_2 g^{-1} h^{-1}), in *P. ochraceus* tested in 12 combinations of color, location and salinity (n = 72).

Table 3.2. Results of a three-way Analysis of Covariance (ANCOVA) of rank-transformed mass-specific oxygen consumption rates (mg O_2 g^{-1} h^{-1}) of wet tissue, for color, location, and salinity (n = 72).

Source	Df	Mean Square	F	Sig.	Adjusted Partial η^2
Color	1	68.439	0.233	0.631	0.019
Location	1	156.246	0.532	0.468	0.043
Salinity	2	372.932	1.271	0.288	0.198
Color • Location	1	24.780	0.084	0.772	0.007
Color • Salinity	2	2.210	0.008	0.992	0.001
Location • Salinity	2	718.343	2.448	0.095	0.367
Color • Location • Salinity	2	714.722	2.436	0.096	0.365
Rank of Mass (Cov.)	1	3994.783	13.614	0.000	0.187
Error	59	293.434			

Table 3.3. Results of a three-way Analysis of Covariance (ANCOVA) of rank-transformed mass-specific oxygen consumption rates (mg O_2 g^{-1} h^{-1}) of dry tissue, for color, location, and salinity (n = 72).

Source	Df	Mean Square	F	Sig.	Adjusted Partial η^2
Color	1	159.023	0.395	0.532	0.029
Location	1	301.096	0.748	0.391	0.054
Salinity	2	631.680	1.568	0.217	0.217
Color • Location	1	0.751	0.002	0.966	0.000
Color • Salinity	2	5.739	0.014	0.986	0.002
Location • Salinity	2	582.166	1.446	0.244	0.201
Color • Location • Salinity	2	1556.801	3.866	0.026*	0.498
Rank of Mass (Cov.)	1	297.055	0.738	0.394	0.012
Error	59	402.741			

Ammonia Excretion

Preliminary assays were conducted to yield two standard curves: one each for the two separate times of data collection for this study. These standards had coefficients of determination (r^2) of 0.935 and 0.973, respectively, and can be seen on the standard curve charts in Figures 3.2 and 3.3.

A three-way ANOVA (n = 34) did not detect a significant difference among any of the main factors. The mean concentrations for the three factors analyzed are presented in Table 3.4 and results of the three-way ANOVA can be seen in Table 3.5. Size was not used as a covariate in the analysis because a preliminary ANCOVA found size to be independent of ammonia excretion ($F_{1,21} = 0.544$, $p > 0.05$).

Although non-significant, a trend that can be seen in these data is that overall means show the highest rates for ammonia excretion at the hyposaline condition (22 psu). Additionally, a trend can be seen that the purple as well as the orange open coast individuals have the lowest ammonia excretion rates at the hypersaline condition (38 psu).

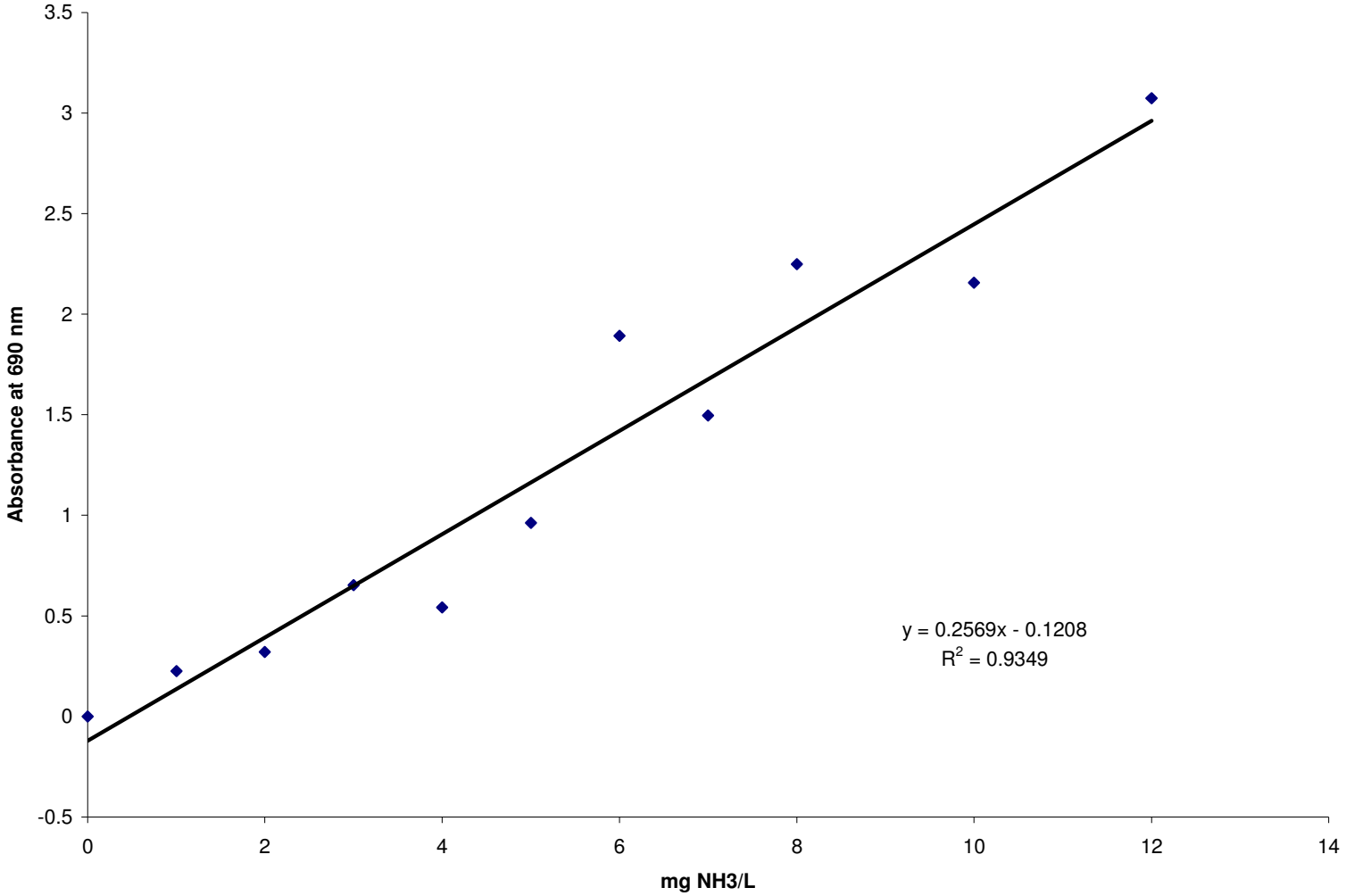

Figure 3.2. Standard curve of concentrations of ammonia that were analyzed by spectrophotometry at 690 nm, produced on July 7, 2006. Slope of standard and r^2 values are presented in the figure.

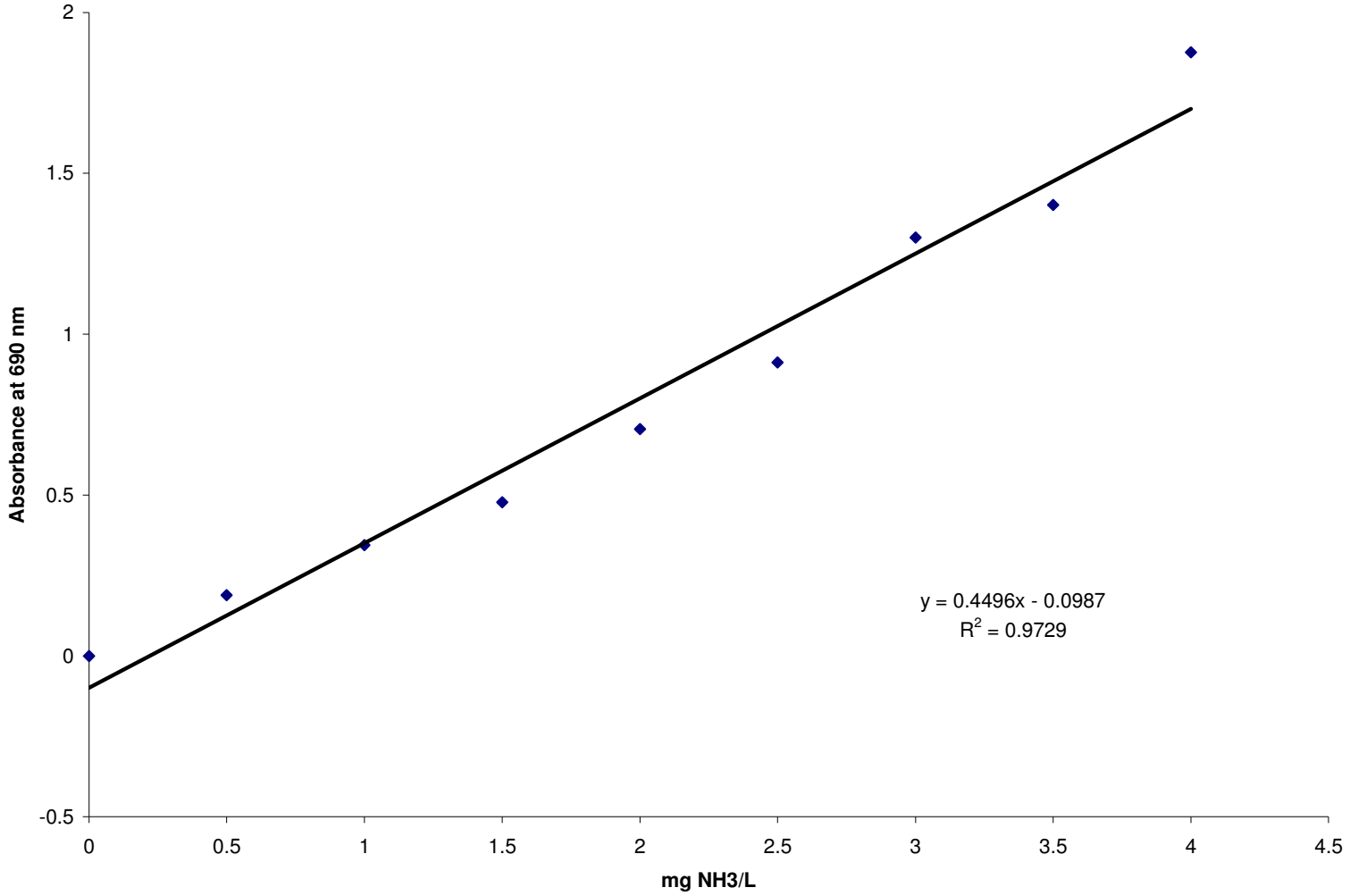

Figure 3.3. Standard curve of concentrations of ammonia that were analyzed by spectrophotometry at 690 nm, produced on August 8, 2006. Slope of standard and r^2 values are presented in the figure.

Table 3.4. A comparison of means (± 1 S.E.) among groups for mass specific ammonia excretion (µg NH_3 L^{-1} g^{-1}(wet) h^{-1}) in 12 combinations of color, location, and salinity (n=34).

Color	Location	Salinity (psu)	N	Mean ± 1 S.E. (µgNH$_3$ L^{-1} G^{-1} H^{-1})
Orange	Open Coast	22	3	0.389 ± 0.150
		30	3	0.157 ± 0.108
		38	3	0.036 ± 0.036
	Inland Straits	22	3	0.184 ± 0.115
		30	2	0.138 ± 0.069
		38	3	0.151 ± 0.049
Purple	Open Coast	22	3	1.072 ± 0.994
		30	3	0.156 ± 0.106
		38	3	0.010 ± 0.006
	Inland Straits	22	3	0.291 ± 0.069
		30	2	0.160 ± 0.088
		38	3	0.126 ± 0.059

Table 3.5. Results of a three-way Analysis of Variance (ANOVA) of rank-transformed ammonia excretion rates for color, location, and salinity (n=34).

Source	Df	Mean Square	F	Sig.	Adjusted Partial η^2
Color	1	0.134	0.461	0.504	0.021
Location	1	0.137	0.471	0.500	0.021
Salinity	2	0.545	1.881	0.176	0.146
Color • Location	1	0.070	0.241	0.628	0.011
Color • Salinity	2	0.158	0.547	0.587	0.047
Location • Salinity	2	0.306	1.054	0.365	0.087
Color • Location • Salinity	2	0.083	0.287	0.753	0.025
Error	22	0.290			

Self-Righting

To evaluate the effects of salinity, color, and location on self-righting times in *P. ochraceus*, a three-way ANOVA was conducted. These data did not meet the assumptions of normality and homoscedasticity, so a rank-transformation was performed before the statistical analysis was conducted. Size was used initially as a covariate in an ANCOVA model, but was found non-significant ($F_{1,23} = 0.005$, $p > 0.05$). Subsequently an ANOVA model was used to analyze these data.

A comparison of mean self-righting times of the groups for the three factors analyzed (color, location and salinity) can be seen in Table 3.6 and Figure 3.4. There was a significant difference in self-righting times for color ($F_{1, 24} = 9.93$, $p<0.05$), with orange having faster turnover times than purple. There was also a significant difference for location ($F_{2, 24} = 4.837$, $p<0.05$), with Inland Straits populations having faster righting times than open coast ones. Salinity also was significant ($F_{1, 24} = 5.818$, $p<0.05$), with individuals exhibiting faster self-righting times at 30 psu than at 40 or 20 psu. The results of the three-way ANOVA test for these data can be seen in Table 3.7.

A post-hoc Tukey test of multiple comparisons was performed for salinity, and a significant difference was found in self-righting times between 20 and 30 psu (20 psu: 1920 ± 493 sec; 30 psu: 474 ± 128 sec., $p < 0.05$) as well as between 30 and 40 psu (30 psu: 474 ± 128 sec; 40 psu: 1323 ± 515 sec., $p < 0.05$).

Table 3.6. A comparison of means (± 1 S.E.) between groups for self-righting times (seconds) in 12 combinations of color, location, and salinity (n = 36).

Color	Location	Salinity (psu)	N	Mean ± 1 S.E. (seconds)
Orange	Open Coast	20	3	653 ± 481
		30	3	201 ± 50
		40	3	338 ± 112
	Inland Straits	20	3	876 ± 442
		30	3	503 ± 209
		40	3	2057 ± 817
Purple	Open Coast	20	3	3600 ± 0
		30	3	304 ± 123
		40	3	802 ± 376
	Inland Straits	20	3	2551 ± 1049
		30	3	887 ± 130
		40	3	2095 ± 755

Table 3.7. Results of a three-way Analysis of Covariance (ANOVA) of rank-transformed self-righting times as a function of color, location, and salinity (n = 36).

Source	Df	Mean Square	F	Sig.	Adjusted Partial η^2
Color	1	622.491	9.934	0.004*	0.250
Location	2	303.125	4.837	0.017*	0.245
Salinity	1	364.573	5.818	0.024*	0.166
Color • Location	2	156.752	2.501	0.103	0.147
Color • Salinity	1	10.614	0.169	0.684	0.006
Location • Salinity	2	141.002	2.250	0.127	0.135
Color • Location • Salinity	2	48.082	0.767	0.475	0.051
Error	24	62.663			

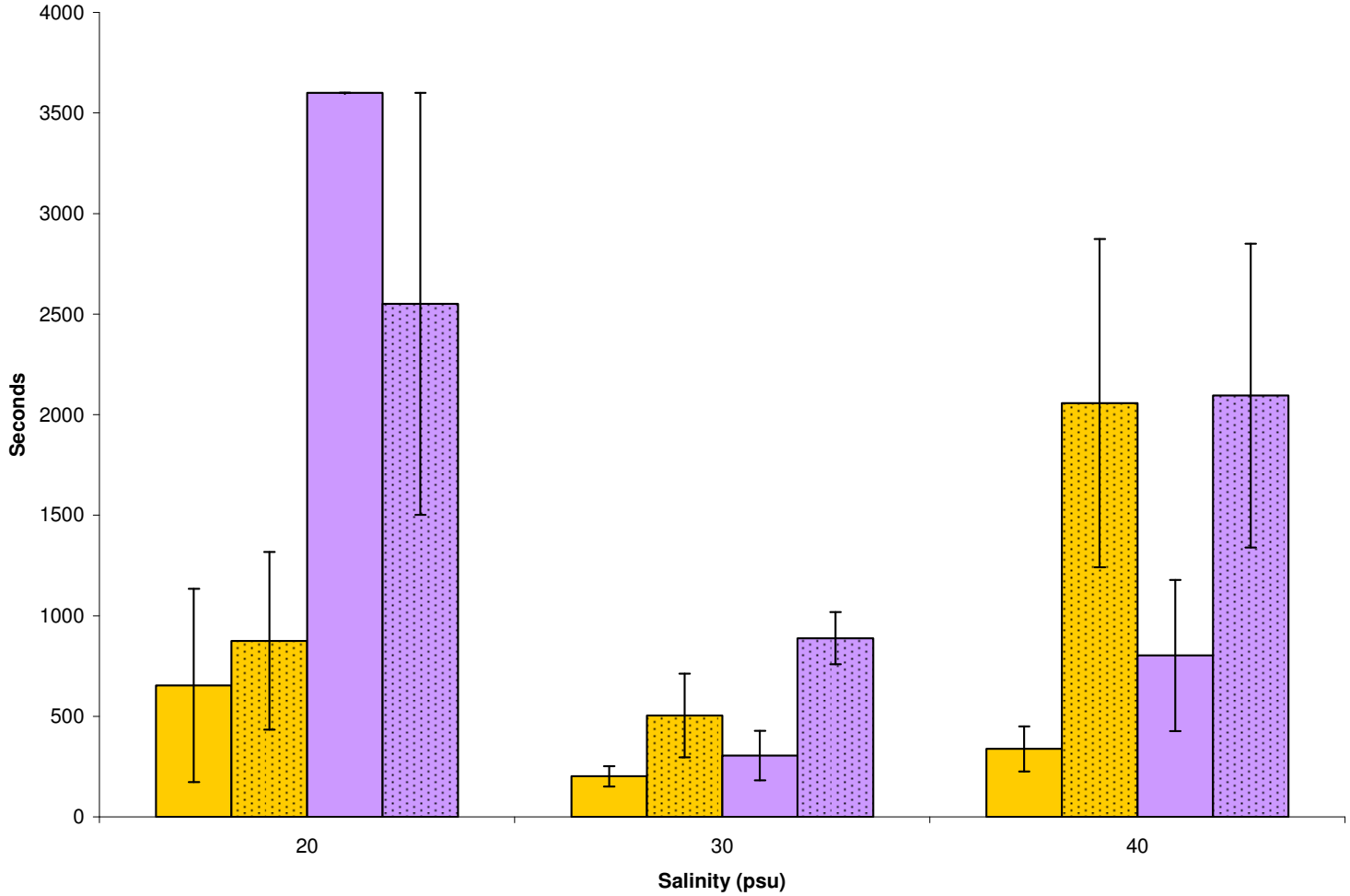

Figure 3.4. A comparison of the mean self-righting times (sec.) of different colors, locations and salinities. Means of orange-open coast individuals ( ), orange-inland straits individuals ( ), purple open coast individuals ( ), and purple inland straits individuals ( ), are reported ± 1 S.E. (n = 36).

CHAPTER FOUR

DISCUSSION

Aerobic Respiration

No significant differences were found among the mean metabolic rates in the 12 combinations of color, location, and salinity for wet mass. In addition, no significant differences were identified in these combinations for dry mass metabolic rates. However, there was a three-way interaction among color, location and salinity, but an analysis of the simple main effects of this interaction showed no significance ($p > 0.05$).

Although these data point to the fact that color, location and salinity are not correlated with metabolic rates, I suspect that under more extreme conditions, such as greater salinity stress ($> \pm 8$ psu from environmental) or a greater sample size to reduce the variance, the effects of color, salinity or location may have been detected.

Other studies, dealing with the physiology of intertidal organisms, have also failed to show that location or physical environment significantly effect metabolic rates of *Pisaster ochraceus.* Dalhoff *et al.*(2002) examined ecologically important, rocky intertidal invertebrates from communities with distinct physical and oceanographic characteristics on the wave exposed coast of Oregon. Although other invertebrate species in the same study, such as the mussel, *Mytilus californianus,* the barnacle, *Balanus glandula,* and the snail, *Nucella ostrina,* were found to have significant differences in metabolic rates between the two environments in several of the experiments, *P. ochraceus* did not.

As mentioned in the introduction chapter of this study, Binyon (1972b) suggests that, in general, echinoderms are not as stenohaline as they were once considered to be.

Members of the phylum Echinodermata are found in salinities ranging from 8 - 46 psu. My results are consistent with the idea that these organisms have incorporated physiological mechanisms that allow them to maintain normal aerobic respiration within certain ranges of salinity. This is in spite of the fact that these organisms are reported to have poor abilities to osmo- and iono-regulate, and lack evidence of excretory systems (Stickle and Diehl, 1987). These mechanisms are probably associated with the ecology of *Pisaster ochraceus* and the environmental pressures associated with life in the intertidal zone.

The physiological mechanisms for this ability to cope with salinity stress is one that is currently undescribed in the literature. Brusca and Brusca (2003) comment on this situation by recognizing that the evidence to date suggests that echinoderms are osmoconformers. They recognize that both water and ions pass relatively freely across the thin body surfaces of echinoderms, and they point out that the tonicity of the body fluids vary with environmental fluctuation. They simply state however, that there appears to be some sort of ionic regulation through active transport, but it is minimal.

If extracellular transport of ions is indeed minimal, then there may also be an influence of intracellular regulation that allows these organisms to maintain aerobic respiration, and essentially a steady state metabolism, in spite of acute changes in salinity within a relatively narrow range

The findings of my aerobic respiration study suggest that salinity, at least in the range of ± 8 psu with respect to the acclimation salinity has no effect on the aerobic respiration of different color morphs of *P. ochraceus* from the open coast and inland straits.

Ammonia Excretion

Results of the analysis of ammonia excretion rates also showed no significant differences between the factors analyzed. This was surprising as salinity has been shown to have a greater effect on ammonia excretion rates than on aerobic respiration rates in other echinoderms (Talbot and Lawrence, 2002).

In most echinoderms dissolved nitrogenous wastes diffuse across the body surfaces, such as the podia and papulae in asteroids (Brusca and Brusca, 2003). The mechanisms by which amino acids are lost from the cells during low salinity exposure include deamination and subsequent excretion of ammonia. However the mechanisms by which amino acids accumulate in the cells during high salinity exposure have not been identified in echinoderms (Diehl, 1986). For echinoderms transferred to altered salinity, the adjustment of intracellular osmolality occurs as ions move into or out of the cells, but the change in osmolality is sustained by the change in free amino acids (Sabourin and Stickle, 1981; Diehl, 1986).

The ammonia excretion in response to salinity of the asteroid, *Luidia clathrata,* is similar to that of *P. ochraceus* (Ellington and Lawrence, 1974). Increased rates of ammonia excretion were observed when these sea stars were transferred from the environmental (27 psu) to an hyposlaline condition (16 psu). My findings show trends that agree with the work of Emerson (1968) who found rates of ammonia excretion to increase when the sea cucumber, *Eupentacta quinquesemita* and the urchin, *Strongylocentrotus droebachiensis*, were introduced into reduced salinities. Similarly Shirley and Stickle (1982) found that the percentage of ammonia in the excreta was significantly lower at 30 psu than at 20 or 15 psu in the sea star, *Leptasterias hexactis.*

Incidentally these six-rayed stars share the same ecological habitats as *P. ochraceus,* and are their principle competitors (Lambert, 2000). Although excretion rates in my study were not significantly different among treatments, increases in ammonia excretion rates were seen in both colors and in both locations as salinities were reduced. In comparison with other studies reviewed, I suggest that at greater salinity stress, these organisms may have indeed exhibited these trends to show significant differences.

No significant differences were found when comparing the group means of ammonia excretion as a function of color or location in this experiment. The effect of reduced salinity on nitrogen excretion rates of echinoderms is thought to be related to the method of exposure. Experiments have shown that abrupt exposure to hyposaline conditions results in initial increases in excretion rates that may later return to control levels (Shirley and Stickle, 1982). This may explain the lack of significance detected among groups of different colors or locations as organisms were all acutely exposed to experimental salinities. Actual times that sea stars were exposed to experimental salinities ranged from 1.95 to 7.00 hrs. The fluctuation of excretion rates may have been affected by the method of exposure due to these variances in chamber times, similar to the fluctuations that were reported by Shirley and Stickle (1982). Non-significant differences between ammonia excretion rates of different groups have also be observed in a 1988 study by Stickle that examined patterns of nitrogen excretion in echinoderms. Stickle found that *P. ochraceus* is one of the most active carnivores with one of the highest total nitrogen excretion rates of the organisms he examined. However, there was no significant difference between the ammonia or urea excretion rates of the sea stars before and after aerial exposure (Stickle, 1988). This in turn had an effect on the internal osmolality of the

organism. Data presented in other experiments as well as my own findings seem to support the idea that *P. ochraceus* may have a wider range of tolerance to salinity stress than had once been thought.

I recognize that errors may have occurred in the methods and analysis of the data collected for this portion of the study. As discussed in the methods chapter, data were analyzed separately and similar results were observed between the pooled data and the individual analysis, with both tests identifying the same factors as significant. This formed the basis for the decision to pool the results from the two separate subsets into one analysis.

As with the aerobic respiration study, I suspect that under more extreme conditions, such as salinity stress greater than ± 8 psu from the environmental salinity, or a greater sample size to reduce the variance, a significant difference for the main effects of color or location may have been detected.

Despite the extremely low concentrations of ammonia in samples, excretion rates of *P. ochraceus* were still found to be significantly affected by acute changes in salinity, but only showed non-significant trends. The data from this study however, do not support the alternative hypothesis that ammonia excretion rates differ significantly between color morphs of *P. ochraceus.* Further experimentation may lead to results that support different conclusions from those made here.

Self-Righting

The results from the analyses of self-righting times for the two color morphs of *P. ochraceus* from different locations revealed significant effects in all three main factors (color, salinity and location). There were no interactions among factors.

The first interesting finding of this study was that organisms tested closer to their environmental salinities (30 psu) had significantly faster self-righting times than organisms exposed to hypo-and hyper-saline experimental salinities (20 and 40 psu, respectively). The results of the post-hoc Tukey test identified a significant difference in self-righting times between organisms tested at 20 psu and 30 psu, and between 30 psu and 40 psu. These results are explained by the fact that 30 psu is the salinity that these organisms were acclimated in and closest to the environmental salinities they were found in. Thus, this suggests that organisms that were stressed the least were capable of faster righting responses. Specimens that were tested at 20 psu and 40 psu all had significantly slower self-righting response times. This could be due to the fact that individuals that are more stressed by their environment may have a lower ability to carry out physiological functions because they may be expending excessive energy carrying out intracellular regulation (Ellington and Lawrence, 1974). Additionally, a decreased ability to conduct behavioral functions such as righting responses may be explained by the turgor pressure caused by an inflow of water across body tissues when organisms are placed acutely in a hyposaline condition. Organisms that were placed in the hyposaline condition (20 psu) took significantly longer to undergo self-righting than the organisms in the other two salinities, and organisms at the hypersaline condition (40 psu) took longer than the ones at the environmental condition (30 psu).

A second interesting finding is that in almost all cases, specimens from the open coast would right themselves faster than ones from the Inland Straits. This could potentially be due to the fact that they are smaller individuals which may contribute to faster self-righting times.

The third finding was that in all cases, and for each condition, orange specimens always had faster mean self-righting times than purple specimens. This was the first of the three experiments to identify color as being associated with a different response. This study was limited as it did not approach questions that address what factors of the color polymorphism that occurs in *P. ochraceus* are related to responses such as rates of self-righting. There are currently no clear answers presented by this study that would identify causes for differences in response rates between the two color morphs examined in this study. These findings lead to several questions that will need to be addressed in future studies.

The self-righting study could have been strengthened by a larger sample size in addition to more consistent methods. A sample size of 36 was not ideal for an experiment such as this with so many uncontrollable variables. Further studies of this sort with larger sample sizes and more precise controls may lead to more conclusive findings. Despite the limitations of this experiment, these data do support the hypothesis that self-righting times differ significantly in specimens from two locations, among different salinities and between different color morphs.

Ecological Application of Results

The reason for *P. ochraceus* not showing significant differences in aerobic respiration in this study could be tied to the ecology of these organisms. When compared with most other rocky intertidal fauna in my study areas, *Pisaster ochraceus* is a large, robust organism that is capable of withstanding several hours of desiccation during low tide cycles. Because of their size, these organisms may not be covered by shade found in tidepools as readily as smaller organisms such as the mussels, barnacles and whelks. Thus, it is possible that *P. ochraceus* has different ranges of salinity stress that it is faced with. Reduced metabolic responses to changing salinities may result in a greater tolerance to acute changes in salinity within a certain range.

The idea that these sea stars have a greater tolerance to salinity stress within a certain range is further supported by the work of Shirley and Stickle (1982) on the sea star, *Leptasterias hexactis*. They found that when *L. hexactis* was placed in various experimental salinities, the highest rate of oxygen consumption was at the environmental salinity of 30 psu and at hyposaline conditions of 20 and 15 psu, oxygen consumption rates were significantly lower. A decrease in oxygen consumption with decreasing salinity has additionally been reported for several other echinoderm species (Giese and Farmanfarmaian, 1963; Shumway, 1977; Sabourin and Stickle, 1981; Stickle and Diehl, 1987) however no physiological mechanisms for this change in physiology has been identified.

The reason why this supports the idea that *P. ochraceus* has a greater tolerance to salinity stress is because it has been shown that under more extreme conditions than my experiments, echinoderms in general do actually exhibit significantly lower rates of

oxygen consumption (Millott, 1967; Shumway, 1977; Sabourin and Stickle, 1981; Stickle and Diehl, 1987; Talbot and Lawrence, 2002). However when stressed at less of an extreme, *P. ochraceus* did not exhibit a significant difference in rates of ammonia excretion. The salinities that I used in my experiment may fall within a range that these organisms may be faced with in their ecosystems, and thus suggests a range of tolerance that has not previously reported.

Conclusions

Although measurements from the three experiments carried out in this study do not all point to differences in responses of the two color morphs, they nonetheless provide some evidence that color and location both have a significant effect on self-righting times at the three salinities tested. Additionally, I found that salinity does not affect respiration rates or ammonia excretion rates differently with regards to color or location within the narrow range of salinities tested.

The results of my study suggest that, within a certain range (± 8 psu), *P. ochraceus* appears to be able to maintain normal aerobic respiration and ammonia excretion. When stressed to greater extremes outside of the range they are able to cope with, such as salinities of ± 10 psu or greater, their basic functions of mobility, such as self-righting, may be reduced.

Suggestions for Further Study

Although salinity was the only environmental variable examined in this study, many other differences are seen between the two locations that show different color frequencies of *P. ochraceus*. Variables that could potentially be tested include temperature, sun and ultra violet (UV) exposure, tidal fluctuations, prey availability, diet, pH of seawater, and wave exposure.

Differences between locations caused by anthropogenic factors can also be identified. Differences between the two locations include greater eutrophication in the Inland Straits due to the large amount of agricultural activity, more pollutants from surface runoff from larger urban populations around the Inland Straits, and habitat degradation due to human trampling of sea star habitats. Studies that examined these effects could potentially identify links to the color differences seen between the two locations.

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