Investigating the Effects of Decreased pH on the Intertidal Shore Crab, Hemigrapsus nudus

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Abstract
Ocean acidification has emerged as an issue for marine organisms, due to increased levels of CO2 in the atmosphere. In particular, invertebrates with calcium carbonate exoskeletons seem to be at risk. Our research investigates the potential effect of decreased pH on the weight of Hemigrapsus nudus, a species of intertidal crab found in Northern California. Over a 6 week period, crabs were maintained in individual containers with recirculating chilled seawater at 12.7°C. The control aquaria was maintained at 8.1 pH, while the experimental aquaria averaged a pH of 7.5. Crabs were weighed at two week intervals, preliminary information shows the control crabs increased in weight, while experimental crabs lost weight. Our results suggest that the decreased pH is possibly affecting a component of calcification needed for strong exoskeleton.

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Investigating the Effects of Decreased pH on the Intertidal Shore Crab, *Hemigrapsus nudus*

Avni Gandhi

Submitted in partial fulfillment of the requirements of the Department of Natural Sciences and Mathematics and the Honors Program Dominican University of California Spring 2017

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III. RESEARCH EXPERIENCE OVERVIEW

For the past three academic years at Dominican University of California, I have been researching ocean acidification and its effects on crustaceans. During this time I have worked closely with Dr. Spain and my initial research group has expanded to include sophomore science majors. For me, research has simultaneously been a lot of work and fun. We have done many different things, from catching crabs on rocky beaches, maintaining crabs in aquaria on campus, and learning how to do water quality tests. We have also visited the California Academy of Sciences in San Francisco, Scripps Oceanographic Institute, and the Birch Aquarium in San Diego. Also, we have developed posters and given oral presentations on and off campus.

I attended the West Coast Biological Undergraduate Research Conference in both Spring 2015 and Spring 2017. As a sophomore, Vanessa Mendoza and I were accepted to give an oral presentation, *Examining the Effects of Ocean Acidification on Exoskeletons*. The presentation was an overview of ocean acidification, ocean chemistry, calcification and exoskeletons, and effects of ocean acidification on crustaceans and bivalves. It was an analysis of preliminary lab discussions integrated with content from journal articles. This spring, I gave an oral presentation based on my thesis research. My senior thesis is an experiment on ocean acidification and its effect on crustaceans’ exoskeleton weight. The initial trial was done in Fall 2015 and the experiment was in Fall 2016. This thesis describes the experiment and includes data on one species of crab.
IV. ACKNOWLEDGEMENTS

My research experience was all the better because of the members of my research group with Dr. Diara Spain. I started this journey as a freshman with Vanessa Mendoza, Shany Kalman, Katie Peterson, Jamie Stockman, and Maurice Brady. Last year, we included Adrienne Davis, Briana Chavez, Madisen Cook, and RJ Francisco. Their hard work was essential for data collection and animal maintenance during the experimental period.

I have spent more time with Dr. Spain than any other professor here. Over the years, she has been my advisor, mentor, and supervisor. While I have not known Professor Doreen Gurrola for very long, I found her insight and suggestions to be helpful. With much gratitude and appreciation, I would like to thank them both because they were instrumental to me finishing my seniors honors thesis.
V. INVESTIGATING THE EFFECTS OF DECREASED pH ON THE INTERTIDAL
SHORE CRAB, *HEMIGRAPSUS NUDUS*

A. ABSTRACT

Ocean acidification has emerged as an issue for marine organisms, due to increased levels of CO$_2$ in the atmosphere. In particular, invertebrates with calcium carbonate exoskeletons seem to be at risk. Our research investigates the potential effect of decreased pH on the weight of *Hemigrapsus nudus*, a species of intertidal crab found in Northern California. Over a 6 week period, crabs were maintained in individual containers with recirculating chilled seawater at 12.7°C. The control aquaria was maintained at 8.1 pH, while the experimental aquaria averaged a pH of 7.5. Crabs were weighed at two week intervals, preliminary information shows the control crabs increased in weight, while experimental crabs lost weight. Our results suggest that the decreased pH is possibly affecting a component of calcification needed for strong exoskeleton.

Keywords: ocean acidification, pH, crabs, exoskeleton, crustaceans, *Hemigrapsus nudus*
B. INTRODUCTION

Climate change encompasses a variety of issues including global warming, sea level rise, and extreme weather events. Evidence of increased carbon dioxide (CO$_2$) emissions has been directly linked to the activities of humans. This is widely considered to be responsible for the current environmental issues of global temperature rise and ocean acidification.

Specifically, ocean acidification (OA) is the lowering of pH levels in the ocean. It is caused by the absorption of CO$_2$ from the atmosphere by the ocean, which includes the uptake of man-created emissions and other greenhouse gases (Gattuso and Hanson, 2011). When the increased atmospheric carbon dioxide is dissolved into the ocean, it combines with water to become a weak acid, carbonic acid (H$_2$CO$_3$), which will further dissolve to form hydrogen (H$^+$) ions and bicarbonate (HCO$_3^-$). HCO$_3^-$ is formed by reacting with carbonate ions, which is a natural base found in the ocean to help neutralize the H$^+$. As the concentration of hydrogen (H$^+$) ions increases, pH decreases which leaves the calcium carbonate saturation state in seawater reduced too. The chemical reaction is shown below.

$$ CO_2 + H_2O + CO_3^{2-} \rightarrow H_2CO_3^{2-} \rightarrow HCO_3^- + H^+ $$

There are three forms of CaCO$_3$: aragonite, calcite, and magnesium calcite. Aragonite is more soluble than calcite, these forms have different saturation depths in the oceans (Fig. 1). This means that calcite is the more stable form of CaCO$_3$ (Whiteley, 2011). However, due to anthropogenic CO$_2$ levels, there is evidence of
both aragonite and calcite depth at the saturation point becoming increasingly deeper. This implies that shallow waters will increasingly be undersaturated of both forms over time due to decreased availability of calcium carbonate, potentially affecting many marine organisms (Feely et al., 2004)

There is rising concern over the impact of increased CO$_2$ on the calcification of skeletons of marine organisms (Ries et. al, 2009). Crustaceans, more specifically crabs, have not been widely researched and recent experiments show variable responses to increased acidification (Spicer et. al. 2006; Small et.al, 2010; Wood et.al, 2008). Their major skeletal component is the protein chitin, typically calcified by the deposition of calcium carbonate (CaCO$_3$). Typically, aragonite and calcite are used by crustaceans (crabs, shrimp, lobsters) to strengthen their exoskeleton. Crustacean exoskeleton is composed of multiple layers, the exocuticle and endocuticle together are called the procuticle (Fig. 2). The procuticle has spaces within these layers where the crab deposits calcium and carbonate ions to go through the process of calcification, to form solid calcium carbonate crystals in the exoskeleton (Perry, 2001). If the crab’s weight decreases this is attributed to decreased deposition and calcification, which also implies that the exoskeleton is weaker too.

This research investigated effects of decreased pH on the exoskeleton of the purple shore crab, *Hemigrapsus nudus*. I hypothesize that a six week exposure to a lower pH will result in a decrease in crab weight, resulting from a decrease in exoskeleton calcification.

![Figure 2: The exoskeleton of a crab](image)
C. MATERIALS AND METHODS

1. ANIMAL COLLECTION

*Hemigrapsus nudus* (Fig. 3) were collected from the intertidal zone at Maverick’s Beach and Pillar Point along the west shoreline access point (Half Moon Bay, CA; 37.495394°N, 122.497911°W). Approval was granted through a scientific collecting permit (D-0013566125-3), from the Department of Fish and Wildlife for the State of California. Specimens were captured with nets by hand on Thursday, September 1, 2017 at low tide between 5:10pm and 7:00pm. There were 8 males with an average carapace width of 2.68 cm and 6 females with an average carapace width of 2.75cm. They were transported to Dominican University of California’s aquatic lab in buckets with seawater taken directly from Half Moon Bay. Animals maintained in a recirculating saltwater system aquaria until weight and carapace measurements were taken, then individuals crabs were separated into control and experimental groups.

2. ANIMAL CARE

The animal care included cleaning, feeding, and water tests. Crabs were fed small (0.5cm³) pieces of squid (United Market, San Rafael CA) twice a week. Individual containers were opened to remove feces daily, while general cleaning of all components occurred twice a week. A variety of tests were done for water quality, specifically: ammonia, phosphate, calcium, alkalinity, nitrate, nitrite, and magnesium. If crabs molted during the six-week period, molts were
removed and weighed; crabs were weighed again as well. Table 1 is the animal care weekly schedule used over the six weeks of the experiment.

Table 1: Animal care weekly schedule

<table>
<thead>
<tr>
<th></th>
<th>SUN</th>
<th>MON</th>
<th>TUES</th>
<th>WED</th>
<th>THURS</th>
<th>FRI</th>
<th>SAT</th>
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</thead>
<tbody>
<tr>
<td>CLEAN AQUARIA</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>FEED</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REMOVE FECES</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>REMOVE FOOD</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>WATER TESTS</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. EXPERIMENTAL SETUP

Artificial saltwater was made from Instant Ocean, with a salinity of 35 ppt, and kept in a large reservoir tank (375L). While the experimental and control crabs were maintained separately, the organization was similar but the experimental components were usually larger in size (Long et.al, 2013). Figure 4 is a labeled picture of the larger components. Also, from Figure 4 the order of water flow can be seen: (A) the conditioning tank to the (B) individual containers through small tubing to the (C) plastic overflow container to the (D) chiller, which then goes back into the conditioning tank. The
conditioning aquaria (A)(75L) is equipped with a filter (Aqueon), a protein skimmer (Aqua C, Maxi-Jet 900), and submerged pump (AquaClear Powerhead 201). The submerged pump sent 12.7°C water to a rectangular plastic container, either the control (44.5L) or the experimental (57.5L), through a series of smaller flexible plastic tubing (7.9mm) at a rate of 463 mL min⁻¹.

There were 10 tubes for the control and 18 for the experimental, these tubes transported water into the smaller individual containers (B). Figure 5 shows a view looking down onto the crabs, the tubing and containers can be seen more easily. Depending on the size of the crab, the containers used were 1.25L or 1.8L, the tubing entered the container through a small hole in the top of the lid. There were additional holes in the upper sides of the containers to maintain the water levels at 0.5L for the 1.25L and 1L for the 1.8L. Water leaving through those holes went from the individual crab containers into the larger plastic overflow container (C). A submerged pump (AquaClear Powerhead 201) sent water down into the chiller (D) (AquaEuroUSA, MC-1/10HP). From there water re-entered the conditioning tank for filtering again. Each individual crab container was labeled with a letter and a number (refer to Figure 5) for crab identification. Either an E (experimental) or C (control) was used, this was paired with a sequential number (examples: E1, E2, C1, C2).
4. MANIPULATION OF CO$_2$ LEVEL IN SEAWATER

Each day for approximately 3 hours the pH was modified from its original pH of 7.97 ($\pm$ 0.1) down to about 6.5. The starting pH was chosen as 7.97 because it was representative of where the species were collected (Long et al., 2013). The lowest value of manipulation, 6.5, was chosen from other research done on crustaceans and their relationship to pH (Small et al., 2010; Wood et al., 2016).

For six weeks, the water in the experimental conditioning aquaria was acidified by bubbling in CO$_2$ gas directly into the conditioning tank from a CO$_2$ cylinder (Cornwall et al., 2015). From there, the water was transported into the individual crab containers through the small water tubes. During the manipulation period, the CO$_2$ gas was turned on and off depending on whether or not the pH had reached the ideal level of 6.5.

The pH was measured by an APEX aquarium sensor (Neptune Systems) and by a pH meter (Scientific Instruments, IQ240). The APEX probe collected data all day at 10 minute intervals from the right corner of the conditioning aquaria (Fig. 6).

![Figure 6: APEX pH data for a 24 hour cycle](image)
In contrast, the pH meter probe was used in multiple locations specifically during the three hour manipulation period in 10 minute intervals. This probe was used in two individual crab containers receiving water from a different pump/tubing from the conditioning aquaria to provide accurate pH values and on the left side of the conditioning aquaria. Figure 7 shows averaged pH values from the three locations.

Figure 7: Combined pH data for individual crab containers

Once the pH reached 6.5, the CO$_2$ was turned off. However, if the pH had decreased to 6.5 significantly before the three hours of manipulation was done, the CO$_2$ was turned off for a little while and turned on again. This allowed the mimicking of an intertidal zone pH. In total, for every 10 minutes during the manipulation four pH data points were collected: two from conditioning tank (left and right sides) and two from individual crab containers (left and right).
5. CRAB WEIGHT MEASUREMENT PROCEDURE

The initial weight and carapace measurement were taken the third day after collection. Thereafter, every two weeks during a total of six weeks, crabs were measured and weighed.

An individual crab was placed in a small container which was put into a freezer for 10 (+/-) 3 minutes, this time variation was dependent on crab size. This process reduced crab mobility and prevented claws from pinching while measurements were being taken. Initial intake data recorded included species and sex identification. Refer to Figure 3 for a picture of a specimen of *Hemigrapsus nudus*. Crab gender was determined by viewing the ventral abdomen, females have a wide abdomen flap, whereas males have a narrow abdomen flap. To further differentiate genders, males have a triangular genitalia structure on their abdomen.

Using a small, flexible ruler the crab’s carapace width and length (cm) were measured. To measure carapace width, the ruler was placed laterally across the body. For measuring carapace length, the ruler measured anterior to posterior distance. Crabs were weighed in grams using a scale (Mettler Toledo, PB602-S/FACT).

6. FINAL WEIGHT MEASUREMENT AND DESICCATION

At the end of the six weeks, final weight and carapace measurements were taken using the standard procedure. Then the crabs were anesthetized and sacrificed by freezing. The final crab weight was determined by desiccating the crabs using a drying oven (VWR, 1510E) (Cameron et.al, 1985). Measurements were taken over the course of three weeks and final dry weight was determined after the dried crabs weights no longer decreased.
D. RESULTS

1. WEIGHT DATA

Each crab was weighed a total of four times over the course of the experiment. The initial measurement, week 0, was the start of the experiment, while week 6 was the end. Table 2 contains weight measurements in two week intervals. The three columns in grey, are data for the crabs in the control group, and the remaining are the experimental group.

Table 2: Six weeks of weight measurements (g) for *Hemigrapsus nudus* (C, control crabs; E, experimental crabs)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>C.a (g)</th>
<th>C.b (g)</th>
<th>C.c (g)</th>
<th>E.a (g)</th>
<th>E.b (g)</th>
<th>E.c (g)</th>
<th>E.d (g)</th>
<th>E.e (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.0</td>
<td>8.75</td>
<td>9.25</td>
<td>11.28</td>
<td>46.3</td>
<td>10.23</td>
<td>10.7</td>
<td>11.44</td>
</tr>
<tr>
<td>2</td>
<td>7.3</td>
<td>7.21</td>
<td>7.03</td>
<td>9.91</td>
<td>47.08</td>
<td>12.72</td>
<td>9.91</td>
<td>8.84</td>
</tr>
<tr>
<td>4</td>
<td>9.11</td>
<td>8.18</td>
<td>9.69</td>
<td>10.1</td>
<td>46.76</td>
<td>9.37</td>
<td>13.81</td>
<td>9.89</td>
</tr>
</tbody>
</table>

The data lies within a range of 7 to 49 grams. However, most data falls between 7 and 14 grams. The outlier, E.b., raises the range due to it initially being a large crab. While overall the crabs weights fluctuated throughout the six weeks, at the end of the experiment, all were within 1.94g of each other, excluding the outlier.
Figure 8 shows Table 2 data in graph form, the crab weight throughout the duration of the experiment in two week intervals. The purple represents the experimental crabs and the red are the control crabs.

Through the graph, it can be seen that all crabs had decreased in weight within the first two weeks, except one experimental crab. Another significant data point is the experimental crab crab that decreased in weight the first two weeks, but had a significant increase, compared to the other crabs, between the second and fourth weeks. Then it decreased again between the fourth and sixth weeks. However, it is still seen that from the start of the experiment to the end of it,
there was an increase in weight. Overall, all crabs had fluctuations in weight. Through a T-test, it is seen that the data is statistically significant.

2. CALCIFICATION DATA

Calcification was calculated with the starting wet weights, ending wet weights, and final dry weights.

\[
(\text{Week 0 Wet Weight} / \text{Final Dry Weight}) \times 100\% = \text{Starting Calcification Percentage}
\]

\[
(\text{Week 6 Wet Weight} / \text{Final Dry Weight}) \times 100\% = \text{Final Calcification Percentage}
\]

\[
\text{Starting calcification} - \text{final calcification} = \text{% change in calcification}
\]

Table 3: Change in calcification over six week period

<table>
<thead>
<tr>
<th>Crab ID</th>
<th>Initial Calcification (%)</th>
<th>Final Calcification (%)</th>
<th>Change in Calcification (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.a</td>
<td>27.34</td>
<td>21.81</td>
<td>-5.54</td>
</tr>
<tr>
<td>C.b</td>
<td>29.56</td>
<td>26.23</td>
<td>-3.33</td>
</tr>
<tr>
<td>C.c</td>
<td>23.20</td>
<td>21.01</td>
<td>-2.19</td>
</tr>
<tr>
<td>E.a</td>
<td>32.84</td>
<td>33.14</td>
<td>0.30</td>
</tr>
<tr>
<td>E.b</td>
<td>28.55</td>
<td>31.02</td>
<td>2.47</td>
</tr>
<tr>
<td>E.c</td>
<td>29.57</td>
<td>27.99</td>
<td>-1.58</td>
</tr>
<tr>
<td>E.d</td>
<td>25.51</td>
<td>24.25</td>
<td>-1.26</td>
</tr>
<tr>
<td>E.e</td>
<td>27.01</td>
<td>30.62</td>
<td>3.61</td>
</tr>
</tbody>
</table>

Table 3 above shows the calculated calcification percentages. All control crabs decreased in calcification, whereas experimental crabs varied. Three out of the five experimental crabs
increased their calcification and two had very little decrease. All three *Hemigrapsus nudus* in the control tank had a decrease by more than 2% of its original calcification percentage, whereas the two experimental crabs that had a decrease were less than 2%. Crab E.d had the lowest decrease in percent calcification, but had a great increase in weight between weeks two and four.
E. DISCUSSION

The goal of this research was to investigate the effects of ocean acidification on a marine crustacean, specifically the intertidal shore crab *Hemigrapsus nudus*. The control group was kept in aquaria at 8.1 pH. The experimental crabs were maintained in water with a 7.9 pH baseline but experienced a daily flux to a 6.5 pH value. The results on crab weight changes indicate that pH does affect these crabs over an intermediate period of time (6 weeks).

The results of measurements from the control crabs, show an increase from the initial wet weight to the final wet weight. However, when the dry weight was used to estimate calcification there was a decrease over time. This is unexpected, since the crabs in this treatment were not considered to be under environmental or physiological stress. It may be that the control crabs were using their resources metabolically in preparation to increase in size while molting. Assuming they were younger due to their smaller size and lower initial weights, growth is more important than maintaining a hard exoskeleton (Small et.al, 2010).

The five crabs in the experimental treatment showed variability in the wet weight measurements, two increased but three decreased in weight. Among this group the calcification increased for some and decreased for other crabs. This indicates that crab weight is effected by a decrease in pH, but exactly why is unclear from our data. Ries (et. al., 2009) and Long (et. al., 2014) showed mixed responses for crustaceans, some species have negative and other species have positive changes in calcification in response to lower pH conditions.

*Hemigrapsus nudus* lives in the intertidal zone, depending on the tidal cycle it may be submerged completely or exposed to air/hiding in a small rocky crevice. While completely aquatic crabs are predicted to experience more stress from ocean acidification crabs in the
intertidal zones and land crabs are still likely to be effected too. All crabs need to molt to grow, thus will need to calcify new exoskeletons. Also, crabs will need to maintain calcification to have skeletal strength for movement as well as protection from predators.

Using the same species in a longer experimental period, 8 weeks, would increase the sample size to provide more data to see if a trend develops. I’d also track the levels of calcium to see how much is readily available for the crabs to potentially use in calcification.

Also, looking at another species found in the same area, possibly *Pachygrapsus nudus*, would allow me to compare the two intertidal species. It would be interesting to see if these species have a similar or different response to the same experimental conditions.
F. REFERENCES


VI. APPENDIX

A. State of California, Department of Fish and Wildlife - Scientific Collecting Permit
PERMIT, AMENDMENTS AND REPORT OF SPECIMENS CAPTURED OR SALVAGED MUST BE IN IMMEDIATE POSSESSION WHILE COLLECTING

SCIENTIFIC COLLECTING IS NOT ALLOWED UNDER THE AUTHORITY OF A SPORT FISHING LICENSE

NEW □ RENEWAL □

When renewing, Report of Specimens Collected or Salvaged MUST BE ATTACHED or application will be returned.

CHECK ONE: Nonrefundable Application Fee (submit now) □ INDIVIDUAL OR ENTITY - $105.58 □ STUDENT - $26.27

CHECK ONE: Permit Fee (submit when notified) □ INDIVIDUAL, ENTITY, OR PI - $315.00 □ STUDENT - $52.79

DO NOT SUBMIT THE PERMIT FEE NOW - You will be required to submit the permit fee if the permit is approved.

Fees include a nonrefundable three percent (3%) application fee, not to exceed $7.50 per item. (Section 700.4, Title 14, California Code of Regulations (CCR)).

BEFORE COMPLETING APPLICATION: Read instructions, permit descriptions, standard conditions, and number authorizations requested or issued. Complete all appropriate sections of the application (presence's section may be required). Type or print clearly.

SECTION 1 - INDIVIDUAL PERMITTEE INFORMATION - Complete only if applying as an individual.

FIRST NAME M.I. LAST NAME GO ID NUMBER (FROM ALDS ISSUED LICENSE)

AFFILIATION * Check here if you want future correspondence mailed to your affiliation

TITLE

PERMITTEE'S MAILING ADDRESS DAY TELEPHONE FAX NUMBER

24 Hour Notice: When collecting marine species anywhere in the state you must notify the Monterey office of the event and location of your collecting activities at least 24 hours prior to commencement of such activities. Please fill out Form 1379f (Notification of Intent to Collect for Scientific Purposes) and fax it to (831) 649-2819 or email to R.7monterey_frontoffice@wildlife.ca.gov.

SECTION 2 - ENTITY PERMITTEE - Complete only if applying as a qualified entity.

Entities include California certified small businesses, aquariums or zoos accredited by the Association of Zoos and Aquariums, museums, California Special Districts, public agencies, non-profit non-governmental organizations, accredited colleges or universities and instructors at accredited colleges or universities.

Proof of such status must be provided with the application. An additional permit fee is required for each PI when the permit is ready to be issued.

ENTITY'S NAME:
Dominican University of California

ENTITY'S MAILING ADDRESS CITY STATE ZIP CODE
50 Acacia Ave San Rafael CA 94901

PRINCIPAL SCIENTIFIC INVESTIGATOR (PI) INFORMATION: Provide the following information and attach a statement of qualifications or resume for the PI.

If you have more than one PI proposed under the entity permit, complete and attach page 8 (make copies if needed). The entity shall submit a non-refundable application fee for each PI.

FIRST NAME M.I. LAST NAME GO ID NUMBER (FROM ALDS ISSUED LICENSE) DAY TELEPHONE E-MAIL ADDRESS

Associate Professor of Biology

diara.spain@dominican.edu

List all employees or volunteers that will be working under the Principal Scientific Investigator named above. Attach a separate list if needed.

An amendment form and fee must be submitted, approved, and returned to you by the Department before you can add or remove employees and volunteers from this list:

FIRST NAME LAST NAME DRIVER'S LICENSE OR DMV ID NUMBER STATE
Katie Peterson Ca
Jamie Stockman Ca
Avni Gandhi Il

FOR DEPARTMENT OF FISH AND WILDLIFE USE ONLY

APPROVED BY DATE APPLICATION FEE TRANSACTION # PERMIT FEE TRANSACTION # APPLICATIONS

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