Salinity Tolerance Investigations: A Supplemental Report for the Carlsbad, CA. Desalination Project

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Executive Summary: The goal of this report is to provide additional data and evaluation on the effects of increased salinity on species considered to be sensitive to environmental stress and those species commonly found in the Zone of Initial Dilution (ZID) of the proposed desalination project. The effects of increased salinity were evaluated using two separate operating parameters: 1) conditions that are expected to be present 95% of the time i.e. 36 parts per thousand (ppt) and 2) low probability historical extremes (up to 40 ppt). High salinity salt-water tanks were setup containing a variety of species kept at salinities between 36 and 40 ppt. Observations included both qualitative and quantitative approaches. Results indicate that species living within the ZID will be able to survive successfully at the proposed normal operating condition and will also tolerate salinities above 40 ppt.

Introduction:

The biological assessment for the purposed desalinization project is contained in Graham, 2004. This report used primary papers in the area of biogeography and marine physiology to conclude that our local species are well adapted to tolerate the increase in salinity that has been predicted to prevail after the inception of the desalination plant.

The purpose of this report is to provide experimental data to help confirm the conclusions of Graham's 2004 report. To accomplish this goal, a two-tried approach was implemented. The initial study was a generalized comparative salinity study of local marine species found within the ZID. A collection of 18 marine species was held in an aquarium that contained a blend of desalination plant concentrate and power plant effluent to obtain salinity equal to the salinity that would occur within the ZID 95 % of the time. These animals were then compared to animals held in ambient conditions. The second study was a salinity toxicity study in which selected species of concern were kept at a much higher salinity to capture the biological effects of predicted historical extremes. For this study, the following species were evaluated and checked for survivability: 1) The Purple sea urchin (*Stronglyocentrotus purpuratus*); 2) Sand dollar (*Dendraster excentricus*), and 3) Red Abalone (*Haliotis rufescens*). These species were chosen due to their known susceptibility to environmental stress.

Methods:

Comparative salinity study: A 110-gallon marine aquarium was used to blend power plant effluent and desalination plant concentrate to obtain a salinity of 36 ppt (+/- 0.4 ppt), which is the salinity that is predicted to occur in the middle of the ZID at least 95% of the time (Jenkins and Wasyl, 2004). The marine aquarium contained several local fishes and invertebrates that are known to exist within the zone of initial dilution (Table 1). Organisms were monitored and evaluated for overall health based on the following qualitative parameters: 1) appearance (coloration, tissue marks or legions); 2) willingness to feed; 3) activity, and 4) gonad production in the urchins. These organisms were compared to a second set of organisms held in a control tank. The quantitative parameters measured were percent weight gain/loss and fertilization success of the Purple sea urchin (*Strongylocentrotus purpuratus*). This species was chosen for the fertilization test, since it is the only species contained in the test tank that has an approved protocol as a bioassay species. The protocol used was the sperm activation test based on Dinnel et al. (1987). An overview of Dinnel et al. (1987) is as follows:

1) Spawn animals by injecting 0.5 M KCL.
2) Wash eggs two to three times.
3) Determine sperm density using a Hemacytometer counting chamber and conversion formula.
4) Determine egg density.
5) Set up test tubes with seawater and sperm at proper concentration for the egg density so that resulting ratio is 200:1 (Sperm to egg) (5 reps per treatment and control.)
6) Add eggs and incubate for 60 minutes
7) Stop experiment by adding Formalin.
8) Calculate percent of fertilized eggs and adjust test response using Abbott's formula.

Start date for the comparative salinity study observations began on 11/3/2003 with the final observation made May 18th 2004 (5 ½ months). The Bioassay on the Purple sea urchin was run on May 18th, 2004.

Table 1. Species and number of individuals of each species maintained and observed in the demonstration aquarium.

	Scientific Name	Common Name	# of individuals
1	*Paralichthys californicus*	California halibut	5 juveniles
2	*Paralabrax clathratus*	Kelp bass	3 juveniles
3	*Paralabrax nebulifer*	Barred sand bass	3 juveniles
4	*Hypsoblennius gentilis*	Bay blenny	5
5	*Strongylocentrotus franciscanus*	Red sea urchin	4
6	*Strongylocentrotus purpuratus*	Purple sea urchin	14
7	*Pisaster ochraceus*	Ochre sea star	3
8	*Asterina miniata*	Bat star	3
9	*Parastichopus californicus*	Sea cucumber	2
10	*Cancer productus*	Red rock crab	2
11	*Crassadoma gigantea*	Giant rock scallop	3
12	*Haliotis fulgens*	Green abalone	3
13	*Megathura crenulata*	Giant keyhole limpet	3
14	*Lithopoma undosum*	Wavy turban snail	3
15	*Cypraea spadicea*	Chestnut cowrie	3
16	*Phragmatopoma californica*	Sand castle worm	1 colony
17	*Anthropleura elegantissima*	Aggregating anemone	4
18	*Muricea fruticosa*	Brown gorgonian	1 colony

Salinity toxicity study: Four separate re-circulating saltwater tanks were assembled in the laboratory. Each tank was adjusted to ambient salinity (33.5 ppt). Once the salinity had stabilized, the following three species were added to each tank in groups of five individuals per species: *Stronglyocentrotus purpuratus* (Purple sea urchin), *Dendraster excentricus* (Sand dollar) and *Haliotis rufescens* (Red Abalone). The salinity in each tank was then increased at a rate of 1 ppt per hour until the tanks reached the desired testing level, each tank being 1 ppt greater in salinity then the next tank. The final salinity range in the four tanks was 37 ppt to 40 ppt. The incremental increase in salinity at a rate of 1 ppt was chosen based on communication with Dr. Scott Jenkins who determined that this would be the greatest increase rate that would occur if the power plant operating conditions would necessitate a decrease in their water demand. All tanks were well oxygenated and ammonia levels were checked. If ammonia levels became high, 100% water changes were preformed. Temperature and salinity data were recorded 3 to 4 times per week over the 19 day test run. Any mortality was also noted. All data were compared to a control tank that held all listed species, but kept at ambient condition.

Results:

Comparative salinity study: All organisms remained healthy throughout the test period at the tested parameter of salinity at 36 ppt. No mortality was encountered and all species showed normal activity and feeding behavior. The appearance of the individuals remained good with no changes in coloration or development of marks or lesions. Gonad production in the Purple sea urchin (*Strongylocentrotus purpuratus*) and the Red sea urchin (*Strongylocentrotus franciscanus*) was evident in both the test tank and the control tank by the observation of successful induced spawning by the injection of 0.5 molar KCL. Fertilization of the Purple sea urchin was also successful. Fertilization averaged 99.2 % over the five replicates compared to an average of 99.4% for the control group. This difference was evaluated using the Tukey-Kramer Multiple Comparisons Test, which indicated that there was no statistical difference (P=0.08) among the control group and the treatment group ran at 33 ppt and 36 ppt. The Red sea urchin was not tested for fertilization success, since no approved standard testing protocol exist.

During the test period, there was no significant difference (P=0.07) in the weight gain of the individuals of all species compared to the individuals in the control tank (Table 2). Weight gain in the fish species more than doubled for the 2 bass species (*Paralabrax clathratus* and *Paralabrax nebulifer*) and the Bay blenny (*Hypsoblennius gentiles*) and nearly doubled for the California halibut (*Paralichthys californicus*), which increased 91.3 %. Invertebrate species had a much smaller percent increase but compared well with the control group. Only one species, the Sea cucumber (*Parastichopus californicus*) had an average weight loss of 2.2%, but this loss was not significantly different then that of the control group.

It should be noted that 4 species were removed from the test tank at the onset of the experimental run. The Red rock crab (*Cancer productus*) was removed as result of predation on the Sand Castle worm (*Phragmatopoma californica*) and attempted predation on the other fish species. Filter feeders, the Giant rock scallop (*Crassadoma gigantea*) and the Brown gorgonian (Muricea fruticosa), were also removed after the realization that 24 hour flow-through capabilities were not possible.

Table 2. Overall condition and average weight gain of species contained in the 36 ppt tank. The data is compared to the control group. Statistical significant is based on P< 0.05.

Scientific Name	Common Name	Avg. % wt. change (grams)	% wt. change (Control group)	Sig.	Appearance and Feeding
Paralichthys californicus	California halibut	91.3	96.9	n/s	Strong
Paralabrax clathratus	Kelp bass	114.3	104.8	n/s	Strong
Paralabrax nebulifer	Barred sand bass	106.8	113.5	n/s	Strong
Hypsoblennius gentilis	Bay blenny	120.0	107.1	n/s	Strong
Strongylocentrotus franciscanus	Red sea urchin	2.8	2.4	n/s	Strong
Strongylocentrotus purpuratus	Purple sea urchin	7.9	7.2	n/s	Strong
Pisaster ochraceus	Ochre sea star	3.8	4.6	n/s	Strong
Asterina miniata	Bat star	2.8	3.1	n/s	Strong
Parastichopus californicus	Sea cucumber	-2.2	2.3	n/s	Strong
Haliotis fulgens	Green abalone	9.6	7.7	n/s	Strong
Megathura crenulata	Giant keyhole limpet	5.1	4.7	n/s	Strong
Lithopoma undosum	Wavy turban snail	3.9	2.4	n/s	Strong
Cypraea spadicea	Chestnut cowrie	0.6	1.0	n/s	Strong
Anthropleura elegantissima	Aggregating anemone	115.9	48.9	n/s	Strong

Note: n/s = not significant and Sig. =Statistical significance

Salinity toxicity study: After 19 days, there were no adverse effects to the Purple sea urchins (*Stronglyocentrotus purpuratus*), San dollars (*Dendraster excentricus*) or the Red abalones (*Haliotis rufescens*) at any of the salinities levels tested (37-40 ppt). Both Sand dollars and Red abalones had 100% survival at all salinity ranges and 100% survival in the control chamber (Table 3). One individual in the Purple sea urchin group died in each of the tested levels including one mortality in the control. Therefore, the adjusted survival rate was also 100% for this species. The elapsed time to the first mortality in the Purple sea urchin group increased as salinity increased, which is counterintuitive and indicates that salinity is not a factor causing sea urchin mortality in the tested salinity range.

General observations during the test run showed that all individuals of the species tested were behaving normally. Feeding was active in the Purple sea urchin and Red abalone groups. Dissection of the gut at the end of the test run for the San dollars indicated that feeding was also occurring. Although these species are not known to move actively, movement was noted and compared well with that of the control group.

Table 3. Results of the salinity toxicity test.

Species observed	Salinity (ppt)	Mortality	Elapsed time to 1st mortality (Days)
H. rufescens	33.5 (Control)	0	N/A
H. rufescens	37	0	N/A
H. rufescens	38	0	N/A
H. rufescens	39	0	N/A
H. rufescens	40	0	N/A
D. excentricus	33.5 (Control)	0	N/A
D. excentricus	37	0	N/A
D. excentricus	38	0	N/A
D. excentricus	39	0	N/A
D. excentricus	40	0	N/A
S. purpuratus	33.5 (Control)	1	1
S. purpuratus	37	1	1
S. purpuratus	38	1	4
S. purpuratus	39	1	4
S. purpuratus	40	1	6

Conclusions: Species found in the Southern California bight have geographical ranges that extend into sub-tropical waters. Sub-tropical water is higher in salinity and temperatures then what is expected to occur during normal operating conditions and historical extremesof the proposed desalination plant (Graham, 2004). Therefore, the conclusion that there should be no significant effect to our local biota seems reasonable. The experiments provided in this supplemental report substantiates this conclusion and indicates that species living within the ZID will show no effect at the proposed normal operating condition and will also tolerate salinities at or below 40 ppt. The Comparative salinity study showed that all organisms remained healthy throughout the 5-½ month test period and the Salinity toxicity study indicated that after 19 days, there were no adverse effects to any if the three species test up to 40 ppt.

Literature Cited:

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