

Microbiological Profile of Pacific Shrimp, *Pandalus jordani*, Stowed Under Refrigerated Seawater Spray

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Introduction

Mechanically refrigerated seawater spray (RSWS) has replaced the traditionally used ice to chill shrimp on some Pacific Northwest fishing vessels. The perceived advantages of this system seem to meet the needs of the time. It eliminates dependence on sometimes scarce and costly ice, it results in savings on fuel cost for hauling the ice, and it reduces the labor cost needed to mix the shrimp and ice.

Seawater composition can be easily modified and the shrimp may be super-chilled to below 0°C in seawater. Roach and Harrison (1954) increased the NaCl concentration to 6 percent, and obtained firmer and more readily peelable shrimp. Seagran et al. (1960) gained 1-2 additional days of storage life by storing pink shrimp at -1.1°C (30°F) instead of at the melting ice temperature of 0°C (32°F). Nelson and Barnett (1973) advocated the addition of CO₂ in seawater for bacteriostatic purposes. Ruello (1974) reported that, in addition to being bacteriostatic, the added CO₂ acidified the seawater and inhibited the black spot development in Australian prawn, *Penaeus plebejus*.

To realize these potentials, however, the system has to overcome several limitations. One of these is its inherent characteristic of exposing the entire content of the fish hold to a common environment of recirculating seawater. Any "hot spot" or poorly refrigerated section of the hold will eventually affect the entire catch. Additionally, new catches of unchilled shrimp will continually be added during the trip, each time creating a temperature fluctuation. Adequate design procedures must be followed to minimize these effects. Kolbe (1979a, 1980) has described shrimp cooling characteristics and workable sprayer designs. In a companion paper, Kolbe (1979b) described a RSWS system design model that can be used to select adequate compressor capacities, chiller performance, and other operating parameters.

Optimum performance of the RSWS system, however, cannot by itself assure

high quality of stowed shrimp. The RSWS may become contaminated from an unclean fish hold or from the shrimp itself. The effect of these factors on the ultimate microbial quality of RSW shrimp is largely unknown.

To answer these questions, we isolated and identified microorganisms from both shrimp and seawater samples taken from two RSWS boats during actual fishing conditions. We also employed a self-contained RSWS unit to conduct two controlled experiments, one onboard a fishing vessel, a second onshore. This paper describes our findings.

Materials and Methods

Sampling

Shrimp and refrigerated seawater (RSW) samples were collected on four occasions. Two of these samples were obtained onboard fishing vessels with RSWS systems. Additional samples were obtained from a self-contained RSWS apparatus installed onboard a research vessel, and on another occasion with the apparatus operated onshore. These experimental conditions are described in more detail below.

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ABSTRACT—Microbial counts of refrigerated seawater, which held shrimp at -1.1°C (30°F) for 3 days, ranged from 8.0×10^2 to 1.5×10^6 per ml depending on the systems we examined. Although *Flavobacterium-Cytophaga sp.* predominated the microbial flora of initial seawater (46 percent), the microbial population in subsequently

refrigerated seawater was dominated by *Moraxella sp.* (96 percent) in one instance and *Pseudomonas sp.* (88 percent) in another. *Moraxella sp.* were the most difficult to eliminate on the fishing vessels, and poorly washed shrimp tended to yield more *Arthrobacter sp.*

Most microorganisms grew poorly in chilled seawater, with the exception of *Pseu-*

domonas sp. Therefore, the initial microbial load of the system or of the shrimp, tended to play a greater role in determining the microbial quality of the stowed shrimp than the growth of microorganisms in chilled seawater.

Under optimum conditions, refrigerated seawater spray can maintain the freshness of the shrimp for 5 days.

Table 1.—Microbial count of RSW samples from RSWS boat trip 1.

Sample no. ¹	Time	Microbial count per ml or g
RSW 1	0730, Day 1	$2.7 \cdot 10^4$
2	1230, Day 1	$8.9 \cdot 10^4$
3	1800, Day 1	$9.6 \cdot 10^4$
4	0655, Day 2	$5.3 \cdot 10^5$
5	1205, Day 2	$3.4 \cdot 10^5$
6	2150, Day 2	$3.0 \cdot 10^6$
7	0705, Day 3	$7.6 \cdot 10^5$
8	2100, Day 3	$2.15 \cdot 10^6$
Shrimp ³ 1	1530, Day 1	$1.9 \cdot 10^2$
2	1145, Day 3	$1.7 \cdot 10^4$
3	1650, Day 3	$5.2 \cdot 10^3$
Fish hold ceiling scraping ⁵	0700, Day 1	$4.5 \cdot 10^8$

¹See Figure 1

²Microbial flora identified and presented in Table 4.

³Shrimp samples taken from sorting tables after seawater washing.

⁴Shrimp sample taken from fish hold 1 day after catch.

⁵After the hold had been cleaned and sanitized.

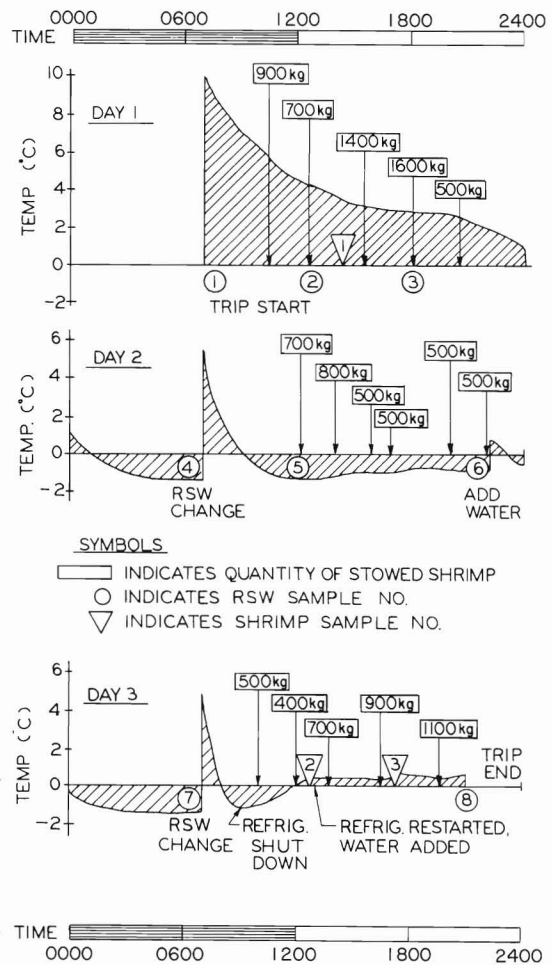


Figure 1.—Trip of RSWS boat 1 and sump temperature. The time and quantity of shrimp stowed, the RSW sample number, and shrimp sample number correspond to those in Table 1.

RSWS Boat 1

The concrete-lined fish hold of a 26 m (86 foot) shrimp trawler was brushed and sprayed with a commercial preparation of sodium hypochlorite solution. After this, about 1,100-1,500 l (300-400 gallons) of seawater containing 4 l of sodium hypochlorite was circulated for approximately 10 minutes. We were not able to determine the exact strength of the sodium hypochlorite solution used.

This boat's fishing trip is schematically presented in Figure 1. Seawater and shrimp samples were taken in sterile plastic bags and frozen immediately by placing on dry ice. After completion of the 3-day trip the samples were delivered

frozen to the laboratory for analysis. The sample numbers shown in Figure 1 correspond to those in Table 1.

The shrimp were thoroughly washed on deck with seawater to remove the bulk of mud and debris, hand sorted, and stowed in the fish hold.

The refrigeration capacity of this vessel appeared marginal. As shown in Figure 1 the spray water temperature which was 5.5°C (42°F) when loading the first batch of shrimp, did not reach below 2.2°C (36°F) for more than 10 hours. The operating temperature of -1.4°C (29.5°F) was reached overnight. Nevertheless, it reached above 4.4°C (40°F) whenever the seawater was changed (once each day). In a few cases, stowed

shrimp temperature increased above 4.4°C (40°F) when new shrimp were loaded on top.

The total catch for the trip was approximately 12,300 kg (27,000 pounds) of shrimp and about 230-370 kg (500-800 pounds) of groundfish.

RSWS Boat 2

The boat 2 fishing trip is illustrated in Figure 2, and the sample numbers here correspond to those in Table 2. The fish hold of this vessel was lined with fiberglass. The refrigeration system of boat 2 appeared slightly more powerful than that of boat 1 and the catch was smaller, with the 3 days total being 7,600 kg (17,000 pounds) of shrimp and 225 kg

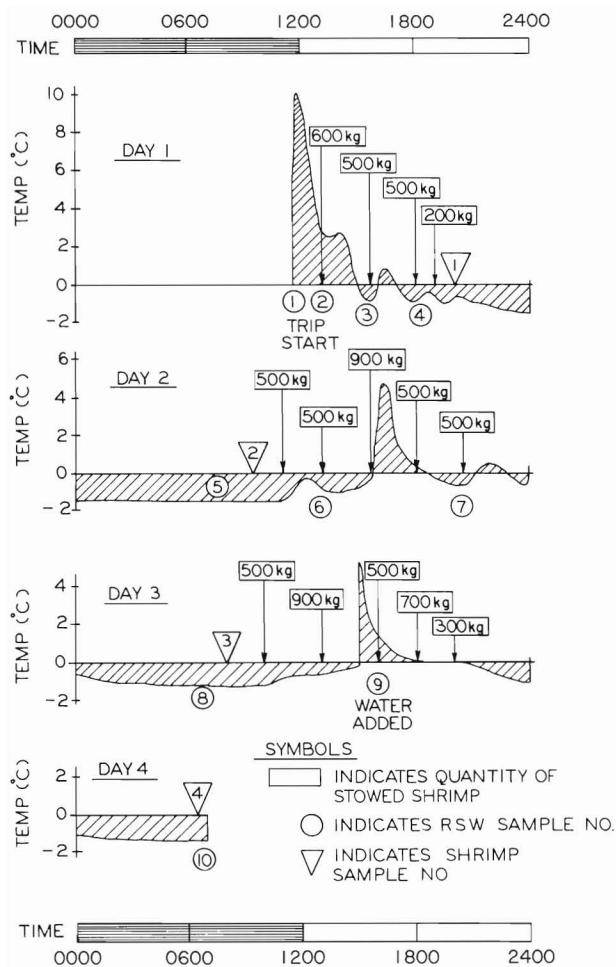


Figure 2.—Trip of RSWS boat 2 and sump temperature. The time and quantity of shrimp stowed, the RSW sample number, and shrimp sample number correspond to those in Table 2.

Table 2.—Microbial count of RSW samples from RSWs boat trip 2.

Sample no. ¹	Time	Microbial count per ml or g
RSW ² 1	1130, Day 1	30
2	1250, Day 1	3.8×10^4
3	1420, Day 1	1.8×10^5
4	1755, Day 1	1.8×10^5
5	0710, Day 2	2.7×10^5
6	1250, Day 2	3.3×10^5
7	1955, Day 2	3.9×10^5
8	0700, Day 3	2.3×10^5
9	1510, Day 3	9.0×10^5
10	0645, Day 4	3.7×10^5
Shrimp ⁴ 1	1950, Day 1	5.5×10^5
2	0920, Day 2	1.0×10^5
3	0740, Day 3	3.7×10^5
4	0645, Day 4	4.9×10^5

¹See Figure 2.

²Seawater at intake.

³Microbial flora identified and presented in Table 4.

⁴Shrimp sample taken from sorting table on deck.

⁵Shrimp samples taken from fish hold.

(500 pounds) of groundfish. Thus RSW temperatures could be maintained at around -1.1°C (30°F) for a greater percentage of the time. On the other hand, the fishermen did not change the RSW during the trip and the shrimp were not washed thoroughly before stowing. The spray coverage was not uniform and the temperature probes placed throughout the hold area revealed several hot spots of 1.7°C (35°F) or above. An iodophor solution of again unknown strength had been circulated through the spray system before filling the sump with fresh seawater.

Sea Trial of Model RSWS Unit

We used a self-contained RSWS unit¹ consisting of a fiberglass insulated tank having dimensions of $1.22 \times 0.61 \times 0.76$ m ($4 \times 2 \times 2.5$ feet) and a refrigeration unit connected to it.

The unit was cleaned thoroughly with a stiff brush, and later Wisk², a liquid

¹This apparatus was loaned by the Utilization Research Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98105.

²Mention of trade names or commercial firms does not imply endorsement by the authors or by the National Marine Fisheries Service, NOAA.

household detergent, was circulated for 30 minutes. After draining, the system was flushed with fresh water. Then a 50 ppm sodium hypochlorite solution prepared from a household bleach was circulated for 30 minutes, after which the tank was drained and allowed to dry overnight. This cleaning and sanitizing regime had been previously tested and found to be effective (Kolbe and Lee, 1980).

The refrigeration unit effectively maintained the RSW temperature at -1.1°C (30°F). The intersecting spray, large droplet nozzles (Bete Fog Nozzle, Inc., Greenfield, Mass.) provided a uniform spray coverage at an average density of 38 l/minute per m^2 (0.94 gpm/foot²).

Table 3.—Contact plate count of fish hold surfaces of RSWs boats awaiting departures.

Boat	Site ¹	Microbial count/cm ²	
		1200 h	1800 h ²
Boat 1	1	TNTC ³	TNTC
	2	51	155
	3	71	>160
	4	28	43
Boat 2	1	14	—
	2	61	—
	3	70	—
	4	>160	—
	5	135	—

¹All were from ceiling reached through hatch covers.

²Cancelled trip of boat 1 offered second sampling opportunity.

³TNTC = too numerous to count.

The 270 kg (600 pounds) of shrimp were put down at the beginning of the trip. This model unit had an efficient straining system and contained no bin board.

Shore Trial of Model RSW System

The self-contained unit described above was installed onshore to extend the holding period beyond the customary 3 days. The unit was cleaned and sanitized as described previously, then filled with a 3.5 percent solution of rock salt and chilled to -1.1°C (30°F). A 180 kg (400 pound) load of freshly caught shrimp was placed in plastic containers, kept chilled onboard a fishing vessel and delivered and placed immediately in the unit. The shrimp was out of water for approximately 5.5 hours by that time. We then operated the unit continuously for 7 days and took periodic samples of RSW and shrimp.

Tests

Temperature

A YSI telethermometer Model 42 with 6 thermister probes (Yellow Springs Instrument Co., Yellow Springs, Ohio) was used to monitor temperatures in various parts of the fish hold and within the experimental unit. The instrument with probes is claimed by the manufacturer to have an absolute accuracy of $\pm 0.6^{\circ}\text{C}$

Table 4.—Microbial flora of seawater and RSW.

Micro-organism (%)	Sea-water ¹	RSW ² of boat 1		RSW of boat 2	
		New	Old	New	Old
<i>Pseudomonas</i>	2.4	6.2	5.2	10.3	5.4
<i>Moraxella</i>	0	64.6	79.3	38.5	50.0
<i>Acinetobacter</i>	2.4	4.6	0	5.1	6.8
<i>Flavo-bacterium-Cytophaga</i>	46.3	16.9	9.5	21.8	16.2
<i>Arthrobacter</i>	24.4	1.5	4.3	23.1	20.3
<i>Lactobacillus</i>	0	0	0.9	0	0
<i>Micrococcus</i>	0	0	0	0	1.4
Unidentified	³ 4.9	6.2	0.9	1.3	0
No. colonies identified	41	65	116	78	74
Count of sample/ml	23	7.7×10^4	1.5×10^6	8.3×10^4	7.3×10^5

¹Fresh seawater at intake.

²New RSW was at the start of the trip before any shrimp was put down. Old RSW was at the end of 3-day trip.

³Resembled *Aerococcus*.

($\pm 1^{\circ}\text{F}$), a reproducibility of $\pm 0.6^{\circ}\text{C}$ ($\pm 1^{\circ}\text{F}$), and a time constant of 7 seconds.

NaCl

The NaCl content in Cl^- concentration, was measured with a Quantab test strip (Ames Co., Elkhart, Ind.).

pH

A Beckman Zeromatic II pH meter was used for pH measurement of RSW samples.

Microbial Count

Microbial number was counted by spread-plating the appropriate dilutions in 0.067 M phosphate buffer, on tryptone-peptone-yeast extract (TPE) agar and incubating aerobically at 25°C for 48-72 hours (Lee and Harward, 1970).

Microbial Identification

The replica-plating scheme was used to identify bacteria to the genus level (Lee and Pfeifer, 1975).

Results and Discussion

Microbial Counts of RSW Obtained at Sea

As described, two trips onboard the RSWs shrimp boats are illustrated in Figures 1 and 2. The temperature of RSW in the sump, monitored continu-

ously during the entire trip, is superimposed on each graph. The time and quantity of shrimp loadings and the time when either the RSW or shrimp samples were taken, are also indicated.

Tables 1 and 2 show the microbial counts of the samples obtained from these trips. The microbial load of seawater measured during the second trip was 30/ml but it increased quickly to $7.7 \times 10^4/\text{ml}$ in RSWs boat 1 and to $8.3 \times 10^4/\text{ml}$ in boat 2 even before the shrimp was introduced to the system. The counts of the final RSW samples at the end of the trips were 1.5×10^6 and $7.3 \times 10^5/\text{ml}$, respectively.

The increase in counts of approximately 10 times in RSW during the 3-day trip was much smaller compared with the sudden increase in counts from 10^1 to 10^4 when seawater was initially recirculated. The recirculating seawater seemed to have been quickly contaminated by inadequately cleaned fish hold surfaces, debris remaining in pipes, and wooden bin boards.

In a separate experiment, we took contact plate counts of fish hold surfaces of the two boats awaiting departure in the harbor and the results are presented in Table 3. The variety of cleaning procedures intended to reduce the initial microbial loading of RSW had not been successful. We found the foam-type detergent effectively cleaned the smooth surfaces, but cleaning inside the pipes and the wooden bin board were next to impossible. Our recommendation for proper RSW system construction, therefore, placed a greater emphasis on construction materials, a piping system having a minimum of dead ends, and the installation of a cleaning loop as previously recommended by Canadian investigators (Gibbard and Roach, 1976; Kolbe and Lee, 1980).

Microbial Growth in RSW During 3-Day Trips

Microbiological data from two trips onboard fishing vessels are summarized in Table 4. As noted, the RSW microbial count did not increase to more than tenfold after 3 days for either boat 1 or 2. This similarity is remarkable if one considers the differences in boat construc-

tion, the load of shrimp stowed, and the contrasting RSW operations. Boat 1 fish hold was constructed with concrete lining, the refrigeration capacity was marginal, and it carried a greater load of shrimp. At the same time, the shrimp was cleaned more thoroughly and the RSW was changed daily. Boat 2 had a smooth fiberglass-lined fish hold, had a greater refrigeration capacity, and did not land a large load of shrimp. On the other hand, the shrimp was not cleaned thoroughly before being placed in RSWS, and the water was not changed during the entire trip.

Table 4 also shows the identities of microorganisms isolated from RSW at the beginning of the trip, before shrimp had been put down, and those from RSW at the end of the trip. In both boats the microbial flora compositions of the new and the old RSW samples were remarkably similar. This indicated perhaps that the microorganisms initially present had equal selective advantages in the RSW or on the shrimp. Little change in microbial flora of RSW in 3 days could also indicate that, despite shortcomings, the microbial growth was kept to a minimum in both RSWS systems.

Moraxella sp. that predominated the microbial flora of the RSW of boat 1 could have come from the unclean fish hold. We observed that the wooden bin boards yielded nearly a pure culture of *Moraxella* sp. even after being dried in the sun. The *Arthrobacter* sp. found in large proportions from boat 2 RSW could have come from the muddy shrimp. This microorganism of soil origin was more numerous in shrimp reared in earthen ponds (Vanderzant et al., 1970).

Microbial Characteristics of RSW Obtained From A Model System Installed on Fishing Vessel

Microbial counts, as well as the identities of those isolated during a holding trial of shrimp in RSW, are presented in Table 5.

This sea trial was conducted to evaluate the performance of a RSWS system under controlled conditions. The fiberglass-lined tank did not require bin boards. Shrimp was put down only once

Table 5.—Microbial flora of RSW and shrimp sampled at intervals.

Microorganism (%)	RSW sample intervals (hours)								Shrimp (hours)	
	6	1	13	24	37	47	58	68	1	68
<i>Pseudomonas</i>	0	12.3	42.7	31.6	76.1	79.8	87.5	70.9	5.8	12.5
<i>Moraxella</i>	0	10.5	6.1	17.1	7.0	4.0	1.4	10.4	0	0
<i>Acinetobacter</i>	0	5.3	7.3	11.8	4.2	8.1	1.4	4.2	2.9	0
<i>Flavobacterium-Cytophaga</i>	0	7.0	3.7	7.9	2.8	4.0	0	4.2	2.9	9.4
<i>Bacillus</i>	0	3.5	0	1.3	0	0	1.4	0	14.7	6.3
<i>Arthrobacter</i>	100	40.4	24.4	13.2	7.0	3.0	5.6	2.1	35.3	46.9
<i>Staphylococcus</i>	0	3.5	11.0	9.2	0	1.0	0	2.1	2.9	3.1
<i>Micrococcus</i>	0	5.3	2.4	3.9	2.8	0	0	0	17.7	21.9
Others ³	0	12.3	2.4	3.9	0	0	2.8	4.2	17.7	0
No. colonies identified ⁴	5	57	82	76	71	99	72	48	34	32
Count of sample/ml or g	2	230	430	540	960	1,200	1,300	800	1,700	2,100

¹RSW chilled to -1.1°C and recirculated for 10 hours before shrimp was put down.

²Shrimp as landed.

³Others include 10.5 percent yeast for 11 hours RSW but the rest were unidentifiable

⁴All isolated colonies on agar plates were picked for identification

at the beginning of the experiment. The spray pattern and chilling rate were controlled to maintain a constant -1.1°C (30°F). The water, however, was not changed during the 3-day holding experiment.

We also tested, with this unit, our recommended cleaning and sanitizing procedures of brush-cleaning with a liquid household detergent and recirculating 50 ppm chlorine, prepared from a household bleach, for 30 minutes (Kolbe and Lee, 1980).

The RSWS system, before introduction of shrimp, was nearly free of bacteria. The count of RSW before shrimp addition was 2/ml of *Arthrobacter* in pure culture. Limited deck space did not allow us to clean the shrimp as thoroughly as we wanted. This perhaps was reflected in the higher proportions of *Arthrobacter* sp. in earlier RSW samples.

The data however, showed a steady increase in *Pseudomonas* population. The initial RSW, after shrimp addition, contained 12 percent *Pseudomonas* sp. with 40 percent *Arthrobacter* sp. During the 68-hour holding period, the proportion of *Pseudomonas* sp. steadily increased to 80 percent of the total. The genus *Pseudomonas* contains many species known to contribute to fish spoilage (Liston, 1980). Therefore, this shift in microbial population of RSW appears

significant, despite the limited increase in the microbial number, from 230 to approximately 1,000/g in 68 hours.

The microbial count of shrimp remained near 2,000/g throughout, and the population shift was not as noticeable as in RSW.

Other than the aforementioned population shift, the types of microorganisms isolated from RSW and RSW shrimp were those commonly found in seafoods (Lee and Pfeifer, 1977; Liston, 1980). An exception was the presence of staphylococci, which were not detected in RSW samples obtained under more aseptic conditions. Thus, they could have been introduced during sampling.

The Model RSWS System Operated Onshore

Microbial number, pH, NaCl concentration of refrigerated 3.5 percent NaCl solution, and the microbial count of the shrimp placed in it, were monitored for 7 days (Table 6).

The microbial count of the shrimp was $9.1 \times 10^5/\text{g}$ before being placed in the RSWS system. It remained at the same or lower level for 7 days in the saltwater solution kept at a constant -1.1°C . The microbial flora of the shrimp consisted almost exclusively of *Moraxella* sp. and, surprisingly, no *Pseudomonas* sp. was found.

Table 6.—Microbial profile of 7-day trial on model RSWS unit operated onshore.

Time of sampling (hours)	Microbial count		RSW		<i>Moraxella</i> (%) of shrimp flora
	RSW	Shrimp	pH	NaCl(%)	
1	1	30	7.2	3.2	— ²
18 (day 1)	18	7.1×10 ⁴	7.9	3.4	93
42 (day 2)	42	4.4×10 ⁴	8.1	2.7	83
60 (day 3)	60	9.7×10 ⁴	8.2	2.5	92
88 (day 4)	88	1.2×10 ⁵	8.1	2.6	94
101 (day 5)	101	2.1×10 ⁵	8.1	2.3	79
136 (day 6)	136	1.3×10 ⁵	8.1	2.1	96
150 (day 7)	153	2.4×10 ⁵	8.0	2.1	96
					95

¹RSW, before shrimp addition, and shrimp, before being placed in RSWS system.

²Not done.

³The remaining microorganisms belonged to *Arthrobacter*, *Acinetobacter*, and *Flavobacterium-Cytophaga* sp. No *Pseudomonas* sp. was isolated.

⁴Shrimp began to show deterioration.

The microbial load of the simulated RSW increased quickly to 7.1×10⁴/ml upon addition of shrimp, but, beyond that, it did not increase noticeably in 7 days. The pH of the RSW increased gradually from 7.2 to 8.0 and the NaCl concentration decreased slowly to 2.1 percent during the same time period.

The RSWS system was set up onshore to test the system beyond 3 days. But this also created the logistical problem of transferring shrimp from the vessel to the system. The shrimp, although held in plastic containers which were stored in a cool environment, were still 5.5 hours out of water. The shore facility did allow us to exercise more care in sampling. The ratio of water to shrimp turned out to be higher (nearly 1:2) than was typically found on shrimp vessels (boat 2 had an estimated ratio close to 1:5).

These differences and, above all, the serendipitous elimination of *Pseudomonas* sp. could have yielded such remarkable performance data for RSWS. Despite the low microbial level, however,

the shrimp began to look bleached, soft, and showed various signs of deterioration on the 5th day. Their appearance was at the borderline of acceptability on the 7th day.

The microbial counts as well as the compositions of the microbial population were quite different for the four different RSWS systems investigated. These differences, however, were easily attributable to the different operation conditions. The data, nevertheless, clearly pointed out that the RSWS system, when properly operated, can effectively control the growth of microorganisms in shrimp, and that the initial microbiological conditions of the shrimp or RSW will determine the ultimate microbiological quality of the RSWS held shrimp.

Acknowledgments

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