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A PRELIMINARY STUDY OF ANTIBIOTIC SENSITIVITY OF PLANKTONIC BACTERIA FROM CULTURES OF THE BRINE SHRIMP *ARTEMIA FRANCISCANA* KELLOGG

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ABSTRACT

Great Salt Lake-biotype brine shrimp cysts were added to 440-l saltwater cultures maintained at 20°, 25°, and 30° C. A micronized corn-byproduct was added each day as food. Planktonic bacteria found during growth of the brine shrimp were enumerated on Plate Count Agar (Difco). From the 25° and 30° brine shrimp cultures, a total of seven prevalent bacterial types (five gram-negative and two gram-positive) were isolated from throughout the 12-day growth cycle. Eight antimicrobial agents were tested against these bacteria, with chloramphenicol the most effective antibacterial agent overall, followed by erythromycin and carbenicillin. The bacterial density of the culture medium upon cyst addition was 10^6 colony forming units (cfu)/ml. The density increased similarly in all cultures from day 1 to day 3, and continued to increase in both the 25°- and 30°-cultures to between 10^8 and 10^9 cfu/ml at the end of day 12, at which time many brine shrimp had reached sexual maturity. However, the bacterial density in the 20°-culture dropped after day 8, the culture medium became less turbid and the brine shrimp did not reach sexual maturity by day 11. Brine shrimp densities from 8 g of cysts were 2.5 animals/ml (day 11) at 20°, 3.0 animals/ml (day 11) at 25°, and 2.7 animals/ml (day 12) at 30°. Final wet weights from one data set were 5.1 g/l at 20°, 10.2 g/l at 25°, and 8.5 g/l at 30° for 12-day-old brine shrimp. The high initial and 1,000-fold increase in density of planktonic bacteria in both the 25°- and 30°-cultures was concomitant with significant accrual of *Artemia franciscana* biomass.

† † †

Newly hatched nauplii of brine shrimp (*Artemia* spp.) are highly regarded as food for fish and crustacean larvae (Bardach et al., 1972; Leger et al., 1986). Still, there are concerns about the nutritional quality of cysts and the omega-3 fatty acid profiles of unenriched nauplii (Dhert et al., 1990; Hontoria et al., 1989; Webster and Lovell, 1990). Recently, as production of adult brine shrimp has become more practical (Bossuyt and Sorgeloos, 1980; Brisset et al., 1982; Lavens et al., 1985, 1987), there has been interest in raising adults as food for later stages of fish and crustaceans. A manual including the subject of growing adult brine shrimp has been developed (Sorgeloos et al., 1986), and various aspects of growth to adults have recently been reviewed for ponds (Tackaert and Sorgeloos, 1991) and for tank cultures (Lavens and Sorgeloos, 1991).

Advantages of intensive indoor culture of these non-selective filter feeders is that their water can be kept relatively clean compared to those harvested from nature, since water quality and causal agents of disease can be monitored and controlled. This is particularly important in the marine ornamental-fish industry where the introduction of disease into large aquaria through brine shrimp or their water could be very costly from the loss of expensive fish.

Brine shrimp can be grown to adults in only a few weeks, with the mouth size of the predator being the determining factor on whether or not to feed them brine shrimp nauplii, preadults, or adults. Protein content varies in adults from about 40% to 60%, the range perhaps related to their food or stage of development, but these data are equivocal (Leger et al., 1986; Ronsivalli and Simpson, 1987; Sorgeloos et al., 1986; Yashiro, 1987). It is common to use agricultural wastes or byproducts for culturing brine shrimp since they may have a cost advantage over natural, microalgal foods or bacteria (Dobbelier et al., 1980; Robin et al., 1987).

For cultured brine shrimp, essential fatty acids, a primary factor in determining the nutritional value of brine shrimp to their consumers (Leger et al., 1987; Sakamoto et al., 1982; Watanabe, 1987; Watanabe et al. 1978, 1982;), can be manipulated with finishing feeds at presumably any stage of their development. Manipulation of the nutritional content of foods in cul-

ture systems is important because brine shrimp on grain-based diets, for example, may be nutritionally poor (Lavens and Sorgeloos, 1991, Lavens et al., 1986), although eicosopentenoic acids can be generated in them from foods that lack them (Ronsivalli and Simpson, 1987). In contrast, in nature, diets of brine shrimp are variable, unpredictable, and uncontrollable.

The use of antibiotics has become widespread in some aquaculture culture/feed situations (Fitt et al., 1992; Grave et al., 1990; Nicolas et al., 1989), and antibiotics have applications in fish and shellfish shipping-water as well (Braley, 1992; Teo et al., 1990). Previous studies of bacteria associated with brine shrimp include studies of cysts and nauplii and have considered the possibility of harmful bacteria being transmitted to larval fish during feeding (Austin and Allen, 1981; Benavente and Gatesoupe, 1988,1989; Gatesoupe, 1982; Gilmour et al., 1975). High densities of *Vibrio* can develop on brine shrimp nauplii and can become pathogenic by inhibiting their swimming (Gunther and Catena, 1980). One filamentous bacterium, *Leucothrix mucor*, is also pathogenic when growing on brine shrimp (Solangi et al., 1979). And, to control bacterial infections in panaeid larvae, brine shrimp have been used to deliver antibiotics to them (Mohney et al., 1990). The usefulness of certain bacteria as a food for brine shrimp has been studied as well, and for many foods bacteria are needed to make them complete nutritionally (D'Agostino, 1980; Dobbelier et al., 1980; Douillet, 1987; Yasuda and Taga, 1980).

Attention has only recently been drawn to the need to know the composition and population density of bacteria associated with cultures on survival and growth of the target larvae (Nicolas et al., 1989). The expected increase in use of cultured preadult and adult brine shrimp in aquaculture has lead us to study the effects of different temperatures on bacterial growth in brine shrimp cultures, to assess bacterial densities, and to note any obvious effects of these bacteria on brine shrimp growth. A benefit to management of culture system planktonic bacteria is that if uneaten food and wastes can be efficiently mineralized by them, food conversion ratios and water quality would likely be improved. In the interest of finding a means to control planktonic bacterial densities and species composition, should control become desirable in the future, antibiotic-sensitivity tests were performed to identify suitable antimicrobial agents. Another use for antimicrobial agents might be in brine-shrimp shipping or storage water, where to decrease bacterial activity might improve water quality thus prolonging brine shrimp longevity. Preliminary data on antimicrobial agents for bacteria in brine shrimp culture water are thus reported with these objectives in mind.

MATERIALS AND METHODS

Culture system

Brine shrimp, *Artemia franciscana* Kellogg, were cultured in 114 cm by 114 cm ABS plastic-lined tanks filled with culture water medium (NaCl and *Instant Ocean*™ mixture, 3 to 1 by weight, with a salinity of 35 ppt) to a depth of 34 cm (about 440 l). The brine shrimp were cultured at three temperatures (20°, 25°, 30° C) in a growth room cooled to below the temperatures required, with the cultures then heated to the appropriate temperatures with four 250-watt aquarium heaters/culture. The pH of the culture medium ranged from 7.6 to 8.7. To each aerated hatch-tank (a 20-gallon aquarium with 6 gallons of saltwater medium) was added 8 g of Sanders brine-shrimp cysts (Great Salt Lake biotype, Utah) in one bank of experiments, and each temperature experiment was repeated at least one time. In further, additional experiments, 9, 9.5, 10, or 11 g of cysts were added instead of 8 g, and the harvests were not necessarily at exactly 12 days. The results of the final biomass, although not strictly comparable, are nevertheless useful.

The tanks were aerated continuously throughout the growth cycle to maintain an oxygen concentration of 4–6 mg/l (monitored with a YSI oxygen meter) except upon food addition, which caused temporary drops in the oxygen concentration. The brine shrimp were fed 3–5 times a day a micronized corn byproduct mixed with water, strained through a 150 μm screen and stored refrigerated until use. Brine shrimp density was determined by using a turkey baster and drawing 3 or 4 samples from the culture tanks, to a volume of ca 100 ml, which assured that at least 250 animals would be counted in each sample; means were determined from three replicates for each tank. Brine shrimp were usually harvested 12 days (range 10–13) after the addition of nauplii, by draining the entire culture through five-gallon buckets with 600 μm screens in the bottom. After most of the water was drained from the shrimp, they were collected into plastic measuring cups with 600-μm screen bottoms for further draining; when water no longer dripped from the cups, the brine shrimp were then frozen and weighed to the nearest 1/4 pound.

Experimental procedures for bacteria

During the 12-day growth cycle of the brine shrimp, bacteria samples were taken with sterile Pasteur pipettes at the same time each day from the center of each experimental tank, 15 cm below the water surface. Bacterial samples were taken from two different sets of experiments at all three temperatures. These samples were immediately diluted and spread-plated in triplicate onto Petri plates containing the general-purpose complex medium *Plate Count Agar* (Difco) with 35 g/l of NaCl. The plates were incubated at 30° (the

highest temperature at which the shrimp were cultured) for four days and the colonies were then counted.

From the 25°- and 30°-cultures, a total of seven prevalent colony types were selected from throughout the 12-day growth cycle. Pure cultures were then isolated from streak plates and transferred to agar slants. The seven different bacterial types were stained and examined for their gram reaction. A variety of growth studies and biochemical tests were also run. The relationship of each type to oxygen was determined using thioglycolate broth. Bacterial motility was tested using semi-solid agar. A summary of the various characteristics for the seven prevalent types of bacteria is found in Table I. Antibiotic sensitivity testing was then performed on the seven isolates by the Kirby-Bauer method using eight different Difco antibiotic-sensitivity disks (Table II).

RESULTS

Growth of the brine shrimp

Growth of the brine shrimp at the three different temperatures occurred most rapidly at 25° and 30°, while the brine shrimp in the 20°-cultures grew noticeably slower. In the 30°-cultures, brine shrimp grew

Table I. Characteristics of the prevalent planktonic bacterial types in the 25°- or 30°-C brine-shrimp (*Artemia franciscana*) cultures.

Bacterial Types	**1[a]**	**2[b]**	**3[c]**	**4[c]**	**5[c]**	**6[d]**	**7[a]**
Colony color	white	pink	green-yellow	yellow-orange	yellow-orange	white	brown
Morphology	rod	rod	rod	rod	rod	rod	rod
Gram stain reaction	–	–	+	–	–	–	+
Relation to oxygen	aer.	aer.	aer.	aer.	aer.	facult.	aer.
Motility	+	ND	+	+	+	+	+
Growth at:							
0% NaCl	+	–	+	+	+	–	+
3.5% NaCl	+	+	+	+	+	+	+
10.0% NaCl	ND	+	+	+	ND	ND	ND
Growth at:							
25° C	+	+	+	+	+	+	+
30° C	+	+	+	+	+	+	+
37° C	+	+	+	+	+	+	+
Catalase	+	ND	+	+	+	+	+
Oxidase	+	ND	–	–	–	+	+
Sugar fermentation:							
glucose	–	ND	–	–	–	–	+
lactose	–	–	–	–	–	–	–
mannitol	–	–	–	–	–	–	–
Spreader colonies	–	–	–	–	–	+	–

[a]isolated from culture water before addition of food
[b]isolated from culture water sometime after day 5
[c]isolated from culture water from days 10 to 12
[d]isolated from culture water from day 3
ND = not determined; + = positive; – = negative

Table II. Antibiotic sensitivity of the predominant bacterial colonies found during growth of the brine shrimp (*Artemia franciscana*) as determined with Difco diffusion test discs.

Antimicrobial		**Colony Number**						
agent	**(μg)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Penicillin G	(10)	–	–	–	–	–	–	–
Kanamycin	(30)	–	–	–	–	–	–	+
Erythromycin	(15)	++	–	++	++	++	+	–
Cephalothin	(30)	++	–	++	++	–	–	–
Chloramphenicol	(30)	++	++	++	++	+	++	+
Ampicillin	(10)	++	+	–	–	+	–	–
Oxacillin	(10)	–	+	–	–	–	–	–
Carbenicillin	(100)	++	–	++	++	++	–	–

++ = sensitive; + = intermediate sensitivity; – = resistant

more slowly than the 25°-cultures in the last few days before harvest. Those brine shrimp in the 25°-cultures were sexually mature adults (6-7 mm); those in the 30°-cultures were similar in size to those in the 25°-culture; there were no adults in the 20°-cultures in 12 days, and exuviae were abundant. Final brine shrimp densities were 2.5/ml, 3.0/ml, and 2.7/ml at 20°, 25° , and 30° respectively. The wet weights of the harvests were 5.2 g/l, 10.2 g/l and 8.5 g/l in one experiment at 20° , 25° , and 30° (Fig. 1) respectively, based on the entire yield from each tank, not on subsamples. A similar experiment at 20° produced 5.7 g/l of brine shrimp in 12 days. In other experiments, 9.5 g of cysts produced 9.8 g/l of brine shrimp in 10 days at 25°, and 9.5 g/l in 13 days at 25°. And, 9 g of cysts produced 9.2 g/l of brine shrimp in 11 days at 25°. But 10 g and 11 g of cysts produced 12.9 g/l and 11.3 g/l of brine shrimp in 12 and 13 days at 30°, respectively.

Bacterial densities of the brine shrimp cultures

Planktonic bacterial densities at the three culture-temperatures for the 12-day growth experiments (two sets of experiments) are shown for a single trial in Figure 2. The additional experimental group at the

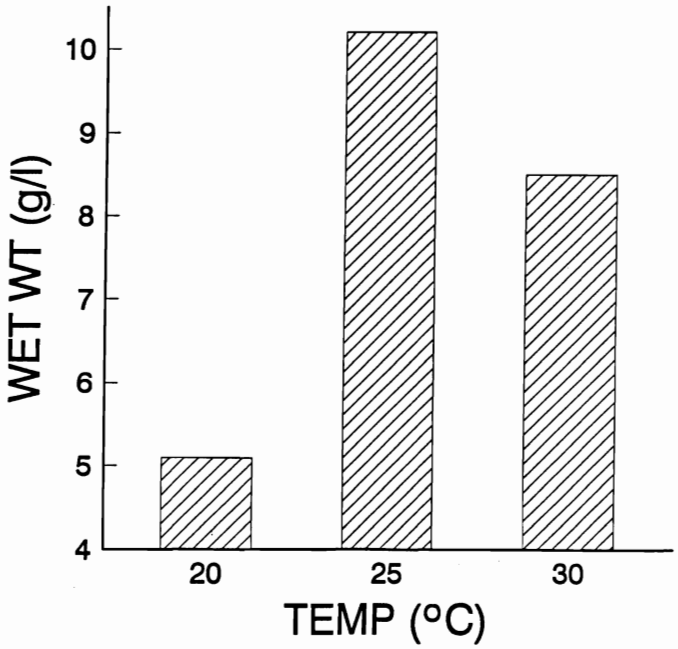

Fig. 1. A histogram of the final wet weight at harvest of *Artemia franciscana* in 20°-, 25°-, and 30°-C cultures.

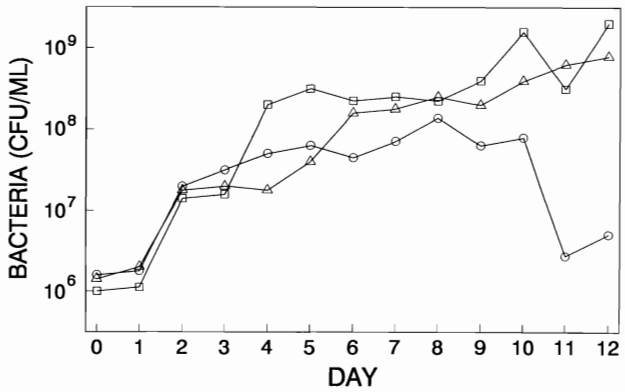

Fig. 2. Development of planktonic bacteria during brine shrimp growth with first food addition at day 1. Temperature in the respective cultures: circles = 20°; squares = 25°; triangles = 30°.

same three temperatures produced similar bacterial densities daily except at 20° for the last two days, in which case the densities were somewhat higher than those shown in Figure 2. In all experiments, the bacterial densities started out similarly at about 1–2 × 10^6 colony-forming units (cfu)/ml and increased tenfold between days 1 and 2 with the initiation of food addition. After day 3, differences in bacterial densities and common colony types could be observed among the 25°- and 30°-cultures. The 20°-culture (Fig. 2) showed the lowest bacterial density. After day 6 at this temperature, the bacterial density leveled off going no higher than 1.4 × 10^8 cfu/ml, and dropped significantly by day 11. The 25°- and 30°-cultures (Fig. 2) had substantially higher bacterial densities, with the 25°-culture being somewhat higher than the 30°-culture. On day 10 the bacterial density of the 30°-culture was about 6.0 × 10^8 cfu/ml, whereas in the 25°-culture the level was 2.0 × 10^9 cfu/ml. In both the 25°- and 30°-cultures, as the bacterial densities steadily increased, the culture water became more turbid from the buildup of wastes which largely remained suspended with continuous aeration. In the 20°-culture the turbidity was lower because of the lower bacterial density and less brine shrimp biomass including feces.

Bacteria characteristics and antibiotic sensitivity

Of the seven bacterial colony types isolated from the 25°- and 30°-brine-shrimp cultures, two were gram-positive rods and five were gram-negative rods (Table I). The two gram-positive rod colony types consisted of a brown type (type 7) found early in the cultures before initiation of feeding of the brine shrimp, and a greenish-yellow colony type (type 3) that was prevalent towards the end of the growth cycle. Both these bacteria are likely members of the genus *Bacillus* (Sneath et al., 1986), but unfortunately no test was made for endospores to further support this conclusion. One of the gram-negative rods (type 5) formed yellowish spreader-colonies (Table I) which sometimes interfered with colony enumeration. Pigmented yellow-orange colonies (types 4, 5) were typical during the later days of the brine shrimp growth in the 25°-tank. Bacterial types 1, 2, and 6 were found early in the growth cycle and continued to be quite prevalent throughout growth of the brine shrimp. While it is somewhat difficult to definitively identify the respective types of these gram-negative bacteria from our data, they all are probably *Pseudomonas*, *Vibrio*, and *Acinetobacter* (Krieg, 1984).

The antibiotic-sensitivity tests showed chloramphenicol to be overall the most effective antimicrobial agent against all of the bacterial colony types tested (Table I). Erythromycin and carbenicillin were effective against more than half of the bacterial isolates tested. All seven isolates were resistant to penicillin G, while intermediate sensitivity was shown for only one isolate each on kanamycin and oxacillin (Table II.).

DISCUSSION

The effect of temperature

During the first few days of growth of the brine shrimp at the three different temperatures, those in the 30°-cultures grew as well as those in the 25°-cultures. However, in the 30°-cultures, good growth was not maintained during the last few days and the water became very turbid. Perhaps at this time some feedings caused oxygen to became momentarily limiting as well, and there may have been a higher aerobic bacterial turnover rate at that higher temperature (the absolute bacterial densities at 25° and 30° were similar however). The results from a single set of experiments showed that for culturing of adult *Artemia franciscana* (from Great Salt Lake cysts, the warm-water biotype) at a density of about 3.0 animals/ml, the highest yield in 12 days of growth occurred at 25°; this data is too limited to state that 25° is the optimum temperature for growth of this biotype, and it should be noted that Vanhaecke et al. (in Lavens and Sorgeloos, 1991) provide data showing the greatest biomass at 30°, with better than 90% survival (Vanhaecke et al., 1984). In a study using axenic (bacteria-free) culture media, it was concluded that the brine shrimp growth rate was greater at 30° than at 25°, but the final biomass was not reported (Hernandorena, 1976). In our own study, a biomass equalling or exceeding that reported for the data set in Figure 1 at 25° occurred by adding more nauplii to tanks as demonstrated in the experiments at 30°. The data provided by Vanhaecke et al. (in Lavens and Sorgeloos, 1991) in which the Utah and San Francisco Bay biotypes were compared, shows the greatest biomass for the Utah biotype at 27.5 and 30° and for the San Francisco biotype at 22.5 and 25°. Clearly there is a wide temperature range (25–31°) at which rapid growth of the Utah biotype occurs.

Brine shrimp in the 20°-culture grew much more slowly than those at the higher temperatures, and they swam slowly even though the oxygen concentration was high. At any given temperature, brine shrimp swim more slowly as oxygen approaches near-depletion levels, but oxygen depletion was not likely at this temperature at any time. The cause of slow growth is likely due to a slower metabolism compared to that at 25° and 30°

The overall lower densities of bacteria at 20° at the end of the growth period suggest that this lower density of bacteria may have played some role in the final lower yield of the brine shrimp in the 20°-cultures. At all temperatures, during the first few days the bacterial densities and the major colony types were similar, many

of them being pigmented. The brine shrimp at the three different temperatures received the same amount of food but did not produce the same growth although the water column became essentially cleared of food soon after each feeding. Precipitation of the food particles might have been more important in the water-clearing than was cropping of the food by the brine shrimp, but we have no data to support this suggestion. On the basis of temperature alone, it is likely that the bacterial turnover rates in the 25°- and 30°-cultures were much greater than in the 20°-culture even though the absolute densities were similar. This would suggest that the brine shrimp were removing the bacteria faster at the higher temperatures. And, they may have been able to crop down the bacteria towards the end of the growth period in the 20°-cultures because of the increase in their size and efficiency of filter feeding in their later stages of growth.

The oxygen concentration of the culture water was higher at the lower temperature as more oxygen dissolves in cooler water, but that positive attribute apparently could not offset the direct effect of the lower temperature on brine-shrimp metabolism. The brine shrimp in all cultures developed a reddish color, but a bright pink-red color indicating that they had been stressed for oxygen and had developed increased levels of hemoglobin over time, was not seen in any of the cultures.

Bacteria as food for brine shrimp

Previous studies with natural foods or food byproducts have shown that the nutritional requirements of brine shrimp are often not met under axenic conditions (D'Agostino, 1980; Douillet 1987). A comparison of dry foods under xenic and axenic conditions showed that bacteria were essential for complete nutrition although the bacterial densities were not determined (Douillet, 1987). Our study suggests that a fortuitous microflora developed and increased from 10^6 to 10^9 cfu/ml over a 12-day period in the presence of significant brine-shrimp growth, with no obvious detrimental effects to the brine shrimp themselves.

The chemical composition of bacteria suggests that they may be highly nutritious and easily digested (Seki, 1964; Zobell, 1946). Indirect evidence for the potential impact of bacteria as food in the Great Salt Lake is that their total biomass far exceeds that of the algal biomass (Post, 1977). A non-living medium for growing brine shrimp axenically to adults is quite complex and, of the requirements, bacteria of xenic cultures likely provide brine shrimp with vitamins, amino acids, and fatty acids (Conklin and Provasoli, 1978; Provasoli and Shiraishi, 1959). Furthermore, brine shrimp may contain bacterial symbionts which aid them in obtaining nutrients from digested food (Post and Youssef, 1977).

Some bacteria are more desirable than others as food for brine shrimp (Douillet, 1987; Yasuda and Taga, 1980). Still, brine shrimp typically have not grown well on diets consisting only of bacteria (Seki, 1965, 1966; Yasuda and Taga, 1980). Brine shrimp from the Great Salt Lake fed halobacteria were smaller than normal and short-lived (Post, 1981). On the other hand, a preliminary report (Intriago, 1992) suggests that a *Flexibacter* not only can serve as a complete food for *Artemia* to adult size, but can aid in digestion of algae through release of exoenzymes in mixed algal-bacterial cultures. It is interesting that of the seven prevalent bacterial types, two of the commonest in our cultures were gram-positive, for certain gram-positive bacteria may release exoenzymes which have been found to be toxic to brine shrimp (Austin and Allen, 1981). Elimination of toxic bacteria from a fortuitous or selected microflora in brine shrimp cultures would be a desirable goal possibly addressed through antibiotic microbial manipulation.

Mixed diets with bacteria

In a study by Douillet (1987), it is interesting that of six foods *Spirulina* was either the best or the worst food for brine shrimp depending on the bacterial flora associated with it. In light of these findings, Lavens et al. (1987) have suggested that evaluations of mixed diets in flow-through systems should be interpreted only as tendencies, since the impact of a favorable or unfavorable microflora is reduced to the extent to which the microflora is removed in the effluent. Although the batch cultures described here are ones in which fortuitous bacteria have been allowed to develop, the system had been in operation for many months and thus there would have been some natural selection of the bacteria to conditions of the cultures over time. That is, those bacteria that remain after a tank is harvested and briefly washed out with tap water will be there to inoculate the next culture. To what extent such bacteria are positive or negative towards water or food quality is not known, but the reasonably high brine-shrimp yields reported here suggest biomass development similar to what others have reported (Lavens et al., 1985; 1987; Rosowski, 1989).

Water quality

Many interacting parameters must be optimized for maintaining water of high quality in batch cultures. It has been suggested that nutritionally-rich diets may give rise to unmanageable bacterial equilibria (Douillet, 1987). If the planktonic bacterial density becomes too high, the water becomes turbid, the oxygen concentration drops, and the brine shrimp grow poorly as happened at 30°in the last few days of our experiments. Figure 2 shows that at the higher temperatures at which the brine shrimp were grown, the bacterial den-

sities were greatest in the last few days of the experiments. However, at low bacterial densities with slower turnover rates, waste materials may not be as completely decomposed as was observed in the 20°-cultures (Fig. 2) where exuviae were conspicuously intermixed with the live brine shrimp at final harvest. The upper and lower limits of planktonic bacterial density acceptability in brine shrimp cultures requires further study, but an increase of from 10^6 to 10^8 cfu/ml in 12 days is a manageable increase in highly aerated cultures.

Past measures to control bacteria in cultures have centered around manipulation of food-particle size (Dobbeleir et al., 1980) and the addition of a selected bacterial microflora (Douillet, 1987). Indirect evidence suggests that the maximum food particle size ingested is 25–10 μm for nauplii and 40–50 μm for adults (Dobbeleir et al., 1980). In our culture system, food was passed through a 150-μm screen; a screen with a much smaller pore size was not used because pore-clogging of the screen made further removal of larger particles impractical. It is likely that the larger food particles not eaten by the brine shrimp accumulated in the cultures and allowed for an increase, with time, in the bacterial density associated with cultures at all temperatures. Of course, fecal material would accumulate during this period and be a readily available substrate for the bacteria as well.

Bacteria in cultures also play an important role in the carbon cycle by decomposing wastes. They appear more active when attached and in aggregates with organic matter because nutrients are high at liquid-solid interfaces (Paerl, 1985; Zobell, 1943; Zobell and Anderson, 1936). The chitinous-encased fecal pellets and the exuviae of brine shrimp are too big to be ingested by brine shrimp, but bacteria in the cultures usually break down this discarded material, and if these bacteria were to become planktonic and associated with particles no larger than about 50 μm , they could become food for the brine shrimp. It is reasonable to speculate that the result of this microbial activity is improved nutrition and water quality if wastes are rapidly mineralized.

Potential use of antibiotics

D'Agostino and Provasoli (1968) tested the tolerance of nauplii and adults to antibiotics. They found that while no single antibiotic was effective in killing all bacteria on brine shrimp, a mixture of penicillin, streptomycin, polymixin and chloramphenicol was effective for the purpose of a one-time treatment for establishing axenic cultures. There was no obvious negative effect on growth of the brine shrimp at concentrations they characterized as "safe," although nauplii "seemed more sensitive" at levels characterized as "toxic." Disinfecting the surface of brine shrimp nauplii has also lead to an improved rate of survivorship of turbot fed such brine shrimp (Benavente and Gatesoupe, 1988; Gatesoupe, 1982). Gilmour et al. (1975) found that a combination of penicillin G and streptomycin improved the effectiveness in killing bacteria on the cyst surface, but penicillin G was ineffective against all seven bacterial isolates from our culture system. A correlation between improved cyst-hatching and antibiotic concentration has been demonstrated at higher hatching densities using chloramphenicol and penicillin-streptomycin (Coleman et al., 1980).

The bacteria found in the culture water of the present study possibly were of different genera than those found on cysts and nauplii by Austin and Allen (1981) but, as indicated earlier, further work is needed to establish the species present. Although the major bacterial isolates proved sensitive to certain of the antibiotics we tested, we have no information on the effects, positive or negative, of these bacteria on the brine shrimp. Also, we cannot speculate on the potential effectiveness of using antibiotics to kept culture conditions from deteriorating, or for shifting the composition of a bacterial flora to a more desirable one since we don't know if the brine shrimp can tolerate antibiotics as a continuous presence. The stability of the antibiotics in highly-bacterialized cultures would have to be assessed as well before remediation procedures could be developed. Furthermore, antibiotics have broad specificity and we don't know which bacteria of a culture may be potentially harmful or useful in the culture system. We know that up to 10^9 cfu/ml were tolerable levels of bacteria in our brine shrimp cultures, and that 10^6 cfu/ml developed only 24 hr after the addition of cysts (Fig. 2) and before the addition of food. The composition of the bacterial community shifted as time passed, and perhaps as soon as new food was added for the brine shrimp.

The literature thus suggests that antibiotics may be considered as management resources when bacteriological problems are addressed in the hatching of brine-shrimp nauplii and culture of adults, and perhaps in their holding- and shipping-water. However, the questions raised in the previous paragraph must be answered before specific experiments on management techniques can be adequately designed. The appearance of resistant strains of gram-negative bacteria in catfish intestinal tracts, and in their water and pond sediments that had been exposed to antimicrobial agents (McPhearson et al., 1991) further suggests that caution must be used in developing therapeutic strategies to minimize the development of antibiotic-resistant strains of bacteria. Swamping a culture system with a desirable bacterium that would out-compete an undesirable species through normal community culture dynamics would be preferable to the use

of antibiotics for this purpose. Given the importance of bacteria in general as food in xenic-culture systems for filter feeders, further attention to optimizing their presence, and directing their species composition and growth is warranted. The recent report by Intriago (1992) of the growth of brine shrimp to adults using only *Flexibacter* strain Inp3 as food suggests this strain as a potential additive for xenic culture-system manipulation.

LITERATURE CITED

Austin, B., and D. Allen. 1981. Microbiology of laboratory-hatched brine shrimp (*Artemia*). *Aquaculture* 26: 369–383.

Bardach, J. E., J. H. Ryther, and W. O. McLarney. 1972. *Aquaculture: the farming and husbandry of freshwater and marine organisms*. New York, Wiley-Interscience: 868 pp.

Benavente, P. G., and F. J. Gatesoupe. 1988. Bacteria associated with cultured rotifers and *Artemia* are detrimental to larval turbot, *Scophthalmus maximus* L. *Aquaculture Engineering* 7: 289–293.

Bossuyt, E. and P. Sorgeloos. 1980. Technological aspects of the batch culturing of Artemia in high densities. *In*: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), *The brine shrimp* Artemia, *Vol. 3, Ecology, culturing, use in aquaculture*. Wetteren, Belgium, Universa Press: pp. 133–152.

Braley, R. D. 1992. Improved method for shipping *Tridacna gigas* seed. *Aquaculture* 102: 193–199.

Brisset, P., D. Versichele, E. Bossuyt, L. De Ruyck, and P. Sorgeloos. 1982. High density flow-through culturing of brine shrimp *Artemia* on inert feeds—Preliminary results with a modified culture system. *Aquaculture Engineering* 1: 115–119.

Coleman, D. E., L. K. Nakagawa, R. M. Nakamura, and E. Chang. 1980. The effects of antibiotics on the hatching of Artemia cysts. *In*: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), *The brine shrimp* Artemia, *Vol. 3, Ecology, culturing, and use in aquaculture*. Wetteren, Belgium, Universa Press: pp. 153–157.

Conklin, D. E., and L. Provasoli. 1978. Biphasic particulate media for the culture of filter-feeders. *Biological Bulletin* 154: 47–54.

D'Agostino, A. 1980. The vital requirements of *Artemia*: physiology and nutrition. *In*: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), *The brine shrimp* Artemia, *Vol. 2, Physiology, Biochemistry, Molecular Biology*. Wetteren, Belgium, Universa Press: pp. 55–82.

D'Agostino, A., and L. Provasoli. 1968. Effects of salinity and nutrients on mono- and diaxenic cultures of two strains of *Artemia salina*. *Biological Bulletin* 134: 1–14.

Dhert, P., P. Lavens, M. Duray, and P. Sorgeloos. 1990. Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*) using w3-HUFA-enriched live food. *Aquaculture* 90: 653–74.

Dobbelier, J., N. Adams, E. Bossuyt, E. Bruggeman, and P. Sorgeloos. 1980. New aspects of the use of inert diets for high density culturing of brine shrimp. *In*: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.), *The brine shrimp* Artemia, *Vol. 3, Ecology, culturing, use in aquaculture*. Wetteren, Belgium, Universa Press: pp. 165–174.

Douillet, P. 1987. Effect of bacteria on the nutrition of the brine shrimp *Artemia* fed on dried diets. *In*: P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (eds.), Artemia *research and applications, Vol. 3. Ecology, culturing, use in aquaculture*. Wetteren, Belgium, Universa Press: pp. 295–308.

Fitt, W. K., G. A. Hesling and T. C. Watson. 1992. Use of antibiotics in the mariculture of giant clams (F. Tridacnidae). *Aquaculture* 104: 1–10.

Gatesoupe, F. J. 1982. Nutritional and antibacterial treatments of live food organisms: the influence on survival, growth rate and weaning success of turbot (*Scophthalmus maximum*). *Annals of Zootechnology* 31: 353–368.

_____. 1989. Further advances in the nutritional and antibacterial treatments of rotifers as food for turbot larvae, *Scophthalmus maximus* L. *In*: M. dePauw, E. Jaspers, H. Ackefors, and N. Wilkins (eds.), *Aquaculture, a biotechnology in progress, Vol. 2*. European Aquaculture Society, Bredene, Belgium: pp. 721–730.

Gilmour, A., M. F. McCallum, and M. C. Allan. 1975. Antibiotic sensitivity of bacteria isolated from the canned eggs of the Californian brine shrimp (*Artemia salina*). *Aquaculture* 6: 221–231.

Grave, K. M., Engelstad, N. E. Soli, and T. Hastein. 1990. Utilization of antibacterial drugs in salmonid farming in Norway during 1980–1988. *Aquaculture* 86: 347–358.

Gunther, D. C., and A. Catena. 1980. The interaction of *Vibrio* with *Artemia* nauplii. *In*: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), *The brine shrimp* Artemia, *Vol. 3, Ecology, culturing, use in aquaculture*. Wetteren, Belgium, Universa Press: 345 pp.

Hernandorena, A. 1976. Effects of temperature on the nutritional requirements of *Artemia salina* (L.). *Biological Bulletin* 151: 314–321.

_____, and S. J. Kaushik. 1981. Ammonia excretion of *Artemia* sp. (Crustacea: Branchipoda) under axenic conditions. *Marine Biology* 63: 23–27.

Hontoria, F., J. C. Navarro, I. Varo, and F. Amat. 1989. Utilisation of *Artemia* cysts in marine larvae cultures: a model of quality evaluation. *Aquaculture Engineering* 8: 127–138.

Intriago, P. 1992. Feeding *Artemia* with bacteria. Abstract for *Aquaculture 92*, Marriott's Orlando

World Center, May 21–25, p. 124, #209.

Krieg, N. R. 1984. *Bergey's manual of systematic bacteriology, Vol. 1.* Baltimore, Maryland, Williams & Wilkins: 964 pp.

Lavens, P., P. Baert, A. De Meulemeester, E. Van Ballaer, and P. Sorgeloos. 1985. New developments in the high density flow-through culturing of brine shrimp *Artemia*. *Journal of the World Mariculture Society* 16: 498–508.

_____, A. De Meulemeester, and P. Sorgeloos. 1987. Evaluation of mono- and mixed diets as food for intensive *Artemia* culture. *In*: P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (eds.), Artemia *research and its applications, Vol. 3, Ecology, culturing, use in aquaculture*. Wetteren, Belgium, Universa Press: pp. 309–318.

_____, P., and P. Sorgeloos. 1991. Production of *Artemia* in culture tanks. *In*: R. A. Browne, P. Sorgeloos, C. N. A. Trotman (eds.), Artemia *biology*. CRC Press, pp. 317–350.

Leger, P., D. A. Bengtson, K. L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. *In*: M. Barnes (ed.) Oceanography and marine biology annual review. Aderdeen Univ. Press: pp. 521–623.

McPhearson, R. M., A. DePaola, S. R. Zywno, M. L. Motes Jr., and A. M. Guarino. 1991. Antibiotic resistance in gram-negative bacteria from cultured catfish and aquaculture ponds. *Aquaculture* 99: 203–211.

Mohney, L. L., D. V. Lightner, R. R. Williams, and M. Bauerlein. 1990. Bioencapsulation of therapeutic quantities of the antibacterial Romet-30 in nauplii of the brine shrimp *Artemia* and in the nematode *Panagrellus redivivus*. *Journal of the World Aquaculture Society* 21: 186–191.

Nicolas, J. L., E. Robic, and D. Ansquer. 1989. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers and turbot larvae: influence of bacteria on larval survival. *Aquaculture* 83: 237–248.

Paerl, H. W. 1985. Influence of attachment on microbial metabolism and growth in aquatic ecosystems. *In*: D. C. Savage and M. Fletcher (eds.), *Bacterial adhesion mechanisms and physiological significance*. New York, Plenum Press: pp. 363–395.

Post, F. J. 1977. The microbial ecology of the Great Salt Lake. *Microbial Ecology* 3: 143–165.

_____. 1981. Microbiology of the Great Salt Lake north arm. *Hydrobiology* 81: 59–69.

_____, and N. N. Youssef. 1977. The procaryotic intercellular symbiont of the Great Salt Lake brine shrimp *Artemia salina*. *Canadian Journal of Microbiology* 23: 1232–1236.

Provasoli, L., and K. Shiraishi. 1959. Axenic cultivation of the brine shrimp *Artemia salina*. *Biological Bulletin* 117: 347–355.

Robin, J. H., C. Le Milinaire, and G. Stephan. 1987. Production of *Artemia* using mixed diets: control of fatty acid content for marine fish larvae culture. *In*: P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (eds.), Artemia *research and its applications*. Wetteren, Belgium, Universa Press: pp. 437–445.

Rosivalli, P. C., and K. L. Simpson. 1987. The brine shrimp *Artemia* as a protein source for humans. *In*: P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (eds.), Artemia *research and its applications, Vol. 3, Ecology, culturing, use in aquaculture.* Wetteren, Belgium, Universa Press: pp. 503–514.

Rosowski, J. R. 1989. Rapid growth of the brine shrimp *Artemia franciscana* Kellogg, in xenic cultures of *Chlorella* sp. (Chlorophyceae). *Aquaculture* 81: 185–203.

Sakamoto, M., D. L. Holland, and D. A. Jones. 1982. Modification of the nutritional composition of *Artemia* by incorporation of polyunsaturaed fatty acids using micro-encapsulated diets. *Aquaculture* 28: 311–320.

Seki, H. 1964. Studies on microbial participation to food cycle in the sea. I. *Journal of the Oceanographical Society of Japan* 20: 122–134.

_____. 1965. Studies on microbial participation to food cycle in the sea. II. *Journal of the Oceanographical Society of Japan* 20: 278–285.

_____. 1966. Studies on microbial participation to food cycle in the sea. III. *Journal of the Oceanographical Society of Japan* 22: 27–32.

Sneath, P. H. A., N. S. Mair, and M. E. Sharpe. 1986. *Bergey's manual of systematic bacteriology, Vol. 2.* Baltimore, Maryland, Williams & Wilkins: 635 pp.

Solangi, M. A., R. M. Overstreet, and A. L. Gannam. 1979. A filamentous bacterium on the brine shrimp and its control. *Gulf Research Reports* 6: 275–281.

Sorgeloos, P., P. Lavens, P. Leger, W. Tackaert, and D. Versichele. 1986. *Manual for the culture and use of brine shrimp* Artemia *in aquaculture*. *Artemia* Reference Center, Ghent, Belgium: 319 pp.

Tackaert, W., and P. Sorgeloos. 1991. Semi-intensive culturing in fertilized ponds. *In*: R. A. Browne, P. Sorgeloos, and C. N. A. Trotman (eds.), Artemia *biology*. Boca Raton, CRC Press, Inc.: pp. 287–315.

Teo, L. H., T. W. Chen, and B. H. Lee. 1990. Packaging of the guppy, *Poecilia reticulata*, for air transport in a closed system. *Aquaculture* 78: 321–332.

Vanhaecke, P., S. E. Siddall, and P. Sorgeloos. 1984. International study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin. *Journal of Experimental Biology and Ecology* 80: 259–275.

Watanabe, T., F. Oowa, C. Kitajima, S. Fugita. 1978. Nutritional quality of brine shrimp, *Artemia salina*, as a living feed from the viewpoint of essential fatty acids for fish. *Bulletin of the Japanese Society of*

Scientific Fisheries 44: 115–1121.

_____, M. Ohta, C. Kitajima, and S. Fugita. 1982. Improvements of dietary value of brine shrimp *Artemia salina* for fish larvae by feeding them w3 highly unsaturated fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries* 44: 1775–1782.

_____. 1987. The use of *Artemia* in fish and crustacean farming in Japan. *In*: P. Sorgeloos, D. A. Bengtson, W. Decleir, E. Jaspers (eds.), Artemia *research and its applications*. Wetteren, Belgium, Universa Press: pp. 373–393.

Webster, C. D., and R. T. Lovell. 1990. Quality evaluation of four sources of brine shrimp *Artemia* spp. *Journal of the World Aquaculture Society* 21: 180–185.

Yashiro, R. 1987. The effect of *Artemia* fed with different diets on the growth and survival of *Penaeus monodon* Fabricius postlarvae. *In*: P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (eds.), Artemia *research and its applications, Vol. 3, Ecology, culturing, and use in aquaculture.* Wetteren, Belgium, Universa Press: pp. 447–457.

Yasuda, K., and N. Taga. 1980. A mass-culture method for *Artemia salina* using bacteria as food. *La Mer* (Bulletin de la Societé franco-japonaise d'Océanographie) 18: 55–62.

Zobell, C. E. 1943. The effect of solid surfaces upon bacterial activity. *Journal of Bacteriology* 46: 39–56.

_____. (ed.). 1946. *Marine microbiology*. Waltham, Mass., Chronica Botanica Company: 240 pp.

_____, and D. Q. Anderson. 1936. Observations of the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. *Biological Bulletin* 71: 324–342.