Objective 5: Regulatory constraints were investigated by personal communications with Gerry Davis, Fisheries Supervisor at the Division of Aquatic and Wildlife Resources (DAWR), Guam Department of Agriculture, Robert Meyer (formerly with the DAWR), and Richard Pyle (Bishop Museum, Honolulu, HI).

Objective 6: Methods of mariculture certification were investigated by personal communications with Gerry Davis of the DAWR and by review of the literature and other mariculture operations.

Objective 7: Cash flow projections were made for a small clownfish hatchery and grow-out facility.

RESULTS:

Objective 1. A few hatcheries are known to be currently producing marine aquarium fish commercially. The largest hatchery is C-Quest, Inc. in Peurto Rico. Another is Reef Propagations, a small basement hatchery in Chicago. A third company is Desert Fisheries in Utah. A fourth hatchery (believed to be the Tropical Marine Centre Ltd.) is located in England. Three other hatcheries are presently operating at a research and development or pilot stage and are supplying small numbers of tank-raised fish to the marine aquarium market. These include a hatchery owned by Red Sea Fish pHarm, Ltd. in Israel, and two closed system hatcheries in the United States owned by SeaPhiz. Four commercial hatcheries formerly produced marine aquarium fish, but are no longer in operation. These include Aqualife Research Corporation in the Bahamas, Dynasty Marine Associates in the Florida Keys, Aquaculture Development A/S in Denmark, and Instant Ocean Hatchery in central Florida.

C-Quest, Inc. completed construction of a 15,000 ft^2 marine fish hatchery in 1991. The hatchery contains twelve hundred 40 gallon aquariums used for broodstock, larval rearing, and research. The facilities have since been expanded to include four hundred 300 gallon outdoor larval rearing and grow-out tanks, nearly doubling the size of the original hatchery. In addition, there are four 25,000 gallon concrete ponds used for large-scale culture of microalgae and grow-out of brine shrimp. Filtration includes biofilters, sand filters, cartridge filters, diatomaceous earth filters, and UV sterilizers.

Commercial production efforts initially focused on clownfish, but have since included two species of goby and three species of dottyback. Several other species have also been cultured at the C-Quest hatchery. The list of species cultured by C-Quest includes the following:

Species	**Common Name**
Amblygobius rainfordi *	Rainford's goby
Amphiprion akallopisis	Skunk clownfish
Amphiprion akindynos	Barrier Reef clownfish

Amphiprion bicinctus	Two-banded clownfish
Amphiprion clarkii	Clark's clownfish
Amphiprion ephippium	Red saddleback clownfish
Amphiprion frenatus	Tomato clownfish
Amphiprion melanopus	Cinnamon clownfish
Amphiprion ocellaris	Common clownfish
Amphiprion percula	Percula clownfish
Amphiprion perideraion	Pink skunk clownfish
Amphiprion sandaracinos	Orange skunk clownfish
Calloplesiops altivelis **	Comet
Chrysiptera cyanea **	Orange-tail Damsel
Gobiodon citrinus	Citron goby
Gobiodon okinawae *	Okinawa goby
Gobiosoma evelynae	Sharknosed goby
Gobiosoma genie	Genie's cleaning goby
Gobiosoma macrodon	Tiger goby
Gobiosoma oceanops	Neon goby
Gramma loreto *	Royal gramma
Gramma melacara *	Black cap basslet
Ophistognathus aurifons *	Yellowheaded jawfish
Premnas biaculeatus	Maroon clownfish
Pseudochromis dutoiti	Neon dottyback
Pseudochromis flavivertex	Sunrise dottyback
Pseudochromis fridmani	Orchid dottyback
Pseudochromis paccagnellae *	Royal dottyback or Bicolor dottyback
Pseudochromis porphyreus *	Magenta dottyback
Pseudochromis springeri *	Springer's dottyback
Pterosynchiropus splendidus **	Mandarinfish

* Occasionally available.
** Not likely to be commercially available in the near future.

In addition to the species listed above, C-Quest has produced one hybrid variety of clownfish that has resulted from five accidental pairings of *Amphiprion ocellaris* and *A. percula*. The hybrids were reared without problems and are called "Percullaris" clownfish. C-Quest has also developed or cultured several color varieties or strains of some of the clownfish species. A bright orange-red *A. percula* and a fast developing dark strain called Early black *A. percula* have been developed. A strain of *A. clarkii* called White-backed *A. clarkii* contains a localized albino area which resembles a white saddle on the fish's back. Another strain called Heart-sleeved tomato clownfish is an *A. frenatus* with a white dot at the base of one or both pectoral fins. C-Quest also produces both white-striped (normal coloration) and blue-striped Cinnamon clownfish (*A. melanopus*), and white-striped (normal coloration) and yellow-striped Maroon clownfish (*Premnas biaculeatus*).

C-Quest is now the largest commercial hatchery for marine aquarium fish in the world. The company is able to sell everything it produces, and is in the process of developing an arrangement to market its fish through a large chain of pet stores in California called Pets Mart (M. Moe, per. comm., 1996). Pets Mart plans to market only tank-raised specimens of marine organisms, instead of wild-caught animals. C-Quest has about 50 wholesale customers and sells about 10,000 fish per month. Dottybacks *(Pseudochromis* sp.) account for only about 150 fish per month, although the company has been getting $15 to $20 per fish (W. Addison, per. comm., 1996). However, wild-caught dottybacks have started becoming more available as new Red Sea collecting stations have recently opened, and the price of these wild-caught dottybacks is much lower than the tank-raised fish. As a result, C-Quest is beginning to have some difficulty in maintaining its price for the dottybacks. The exception is *Pseudochromis dutoiti*, which has not been collected in large numbers from the wild at this time (W. Addison, per. comm., 1996). The owner of C-Quest, Bill Addision, is planning to go to South Africa in the near future to investigate the possibility of a joint venture with an investor there who claims to have successfully cultured Clown triggerfish, Emperor angelfish, Moorish idols, and Powder blue tangs.

Reef Propagations, founded by Joe Lichtenbert in April 1990, is a small (400 square foot) basement operation located in the Chicago, IL area. The company began producing their first salable fish in October 1990, and by the end of 1995 had sold about 50,000 fish for more than $150,000. About one third of these fish were purchased from C-Quest and resold by Reef Propagations (J. Lichtenbert, per. comm., 1996). Sales per week averaged 70 fish for the first two years and rose steadily to over 250 per week (Hoff, In Prep.). Production has recently declined due to technical problems. Total cash outlay at the beginning of 1995 was about $53,000 of which about $10,000 was allocated for research and development (Hoff, In Prep.). This operation now has a positive operating revenue and is operated by 1.5 people with a 45 hour work week.

A company in Utah called Desert Fisheries is using marine geothermal water to raise several species of marine aquarium fish. The company was started in early 1994 and is commercially producing several species of clownfish, including *Amphiprion frenatus*, *A. melanopus*, *A. ocellaris*, and *A. percula*. Desert Fisheries has also successfully reared small quantities of the dottybacks P*seudochromis flavivertex* and *P. fridmani*. At the research stage, the company is spawning the goby *Amblygobius phalaena*, although larval rearing attempts have not been successful at this time. Other species that are targeted for research are the Comet and some angelfish species.

Information obtained from an advertisement in an aquarium magazine indicated that a tropical marine fish hatchery in London, probably Tropical Marine Centre Ltd., has bred the following species in commercial quantities using the artificial sea salt Tropic Marin: *Amblygobius phalaena*, *Amphiprion allardi*, *A. clarkii*, *A. frenatus*, *A. melanopus*, *A. ocellaris*, *A. percula*, *Gobiodon okinawae*, *Gobiosoma evelynae*, *G. oceanops*, *G. puncticulatus*, *Hippocampus comes*, *H. fuscus*, *H. hystrix*, and *Lysmata debelius* (Fire Shrimp).

Red Sea Fish pHarm Ltd. is a manufacturer of artificial sea salt (Coral Reef Sea Salt) and other aquarium products in Israel. This company began an intensive Research and Development program to develop new methods for artificial propagation of marine ornamental fishes in 1994, and has successfully cultured the following species: *Amphiprion bicinctus*, *A. clarkii*, *A. frenatus*, A. *ocellaris*, *Chrysiptera parasema*, *Ophistognathus aurifons*, *Calloplesiops altivelis*, *Pseudochromis fridmani*, *P. flavivertex*, and *Pterosynchiropus splendidus* (Brons, 1995). Red Sea Fish pHarm was the first company to breed two species of fish (i.e., *Pseudochromis fridmani* and *P. flavivertex*) that are indigenous only to the Red Sea. The aquariums and larval tanks at the experimental hatchery are arranged in a closed system equipped with biological filters and protein skimmers. Although the facility is located near the Red Sea, artificial seawater is used in the culture systems. The hatchery has separate facilities for the culture of microalgae and zooplankton. The main goal of the research program is to develop the knowledge and expertise to establish a commercial hatchery producing a wide variety of coral reef fishes. However, the company is also using this research project to promote their artificial sea salt. Red Sea Fish pHarm is presently looking at the economic potential of a commercial hatchery facility (M. Moe, per. comm., 1996). The company believes that all the species they have raised have commercial potential, except for *Chrysiptera parasema* and *Pterosynchiropus splendidus*. The former species does not have a good market value and the latter species has a very low fecundity which limits production (Brons, 1995). The hatchery operation is still at the research and development stage, although the company has reportedly marketed some fish into Europe (S. Brown, per. comm., 1996).

SeaPhiz (formerly EcoActivity, Inc.) is a relatively new company specializing in the manufacture and operation of specialized home aquarium systems (model ecosystems) the company calls Ecotariums and wildlife mariculture systems called ecoCulture facilities. The culture systems are recirculating hatchery systems using algae scrubber technology developed at the Smithsonian Institue. SeaPhiz currently has one ecoCulture system operating in Cleveland, Tennessee and another in Terahaute, Indiana. Species currently cultured in these hatcheries include *Amphiprion clarkii*, a black strain of *A. percula*, *Gobiosoma oceanops*, *Gramma loreto*, *Gramma melacara*, *Hippocampus* sp., *Pseudochromis dutoiti*, *P. flavivertex*, and *P. fridmani*. At the present time, these facilities are just beginning to get up to commercial scale production, with one or two active breeding pairs of each species and a production of about 200 to 250 marketable fish per month (J. Walch, per. comm., 1996). The Arizona-based company is also experimenting with the culture of *Valenciennea strigata*. In addition to the culture of marine aquarium fish, the company is also culturing live coral, live rock, and live sand.

Aqualife Research Corp., located in Walker's Cay, Bahamas, was the oldest company producing marine ornamentals. Until the recent expansion of the C-Quest, Inc. hatchery, it was also the largest marine ornamental fish hatchery in the world. Aqualife Research Corp. closed its hatchery operation in January 1996, after operating on Walker's Cay for the last 12 years. The fish inventory and equipment were sent to Harbor Branch Oceanographic Institute in Fort Pierce, Florida, where a new investor (Dick Williams from

Palm Beach, FL) is beginning to investigate commercial marine aquarium fish culture (G. Waugh, per. comm., 1996).

Aqualife's 25,000 ft^2 hatchery and grow-out facility included a broodstock room containing over 250 broodstock tanks for clownfish and neon gobies (the goby broodstock were kept in a separate room that was air conditioned) and a larval room containing over 40 large tanks (the number of larval rearing tanks was later doubled as production was expanded) and several smaller glass aquariums for larval culture. The live foods area contained an algae laboratory for maintaining small algae cultures, several tanks for hatching brine shrimp, four large transparent cylinders for outdoor algae culture, six 5-ton tanks for growing algae and rotifers, and several large outdoor tanks for brine shrimp. The grow-out area contained over two hundred 300 gallon tanks. Aqualife used saltwater wells which reportedly pumped more than a million gallons of sea water through the hatchery daily. Over 20,000 gallons of microalgae were produced each month. The facility was designed to be capable of producing more than a million fish each year, but the actual production never came close to that amount.

During its peak production in 1989, Aqualife produced and sold over 20,000 fish per month of the following species: *Amphiprion clarkii, A. frenatus, A. melanopus, A. ocellaris, A. percula, A. perideraion, A. polymnus, A. sandaracinos, Gobiosoma oceanops*, and *Premnas biaculeatus* (both Gold-stripe and regular Maroon clownfish). Aqualife's customers included 42 marine aquarium fish wholesalers in the United States, one in Canada, one in England, and one in West Germany. In 1994, Aqualife was producing about 10,000 fish per month.

In the early days of Aqualife (1972-1983), several other species were successfully cultured that never reached commercial production or were commercially produced for only a short time: *Equetus acuminatus, E. lanceolatus, E. punctatus, Gobiosoma evelynae, G. multifasciatum, Gramma loreto, G. melacara, Hypoplectrus gemma, H. unicolor, Microspathodon chrysurus, Opistognathus aurifrons, Pomacanthus arcuatus*, and *P. paru*. Aqualife also produced two hybrids: *Equetus acuminatus x E. lanceolatus*, and *Pomacanthus arcuatus x P. paru*.

Dynasty Marine Associates operated a small hatchery near Marathon, Florida from 1983 to 1995. While it operated, the hatchery produced several species of clownfish, the Neon goby, and seahorses, as a supplement to the main part of the company's business, which is the collection of marine fish, invertebrates, and live rock for the aquarium trade. Although the company no longer raises fish in its own facility, it buys some tank-raised fish from C-Quest, Inc. to include in the inventory of wild-caught fish that Dynasty sells to wholesalers. Species commercially cultured at Dynasty included *Amphiprion clarkii, A. frenatus, A. melanopus, A. ocellaris*, A. *perideraion, Gobiosoma xanthiprora, G. oceanops, Hippocampus sp.*, and *Premnas biaculeatus*. Other species reared at an experimental level included *Amphiprion bicinctus, Amphiprion chrysopterus, Apogon compressus, Bodianus rufus, Chrysiptera cyanea, Equetus acuminatus, Equetus lanceolatus, E. punctatus, Gramma loreto, Microspathodon chrysurus*, and

Opistognathus aurifrons. Dynasty had also produced hybrids on an experimental basis, including *Equetus acuminatus x E. punctatus* and *E. lanceolatus x E. punctatus*.

At one time, the broodstock included about 60 pairs of clownfish and 48 pairs of Neon gobies. Seahorses were raised from wild-caught fish carrying eggs. About two thirds of the broodstock pairs spawned on a regular basis. Each broodstock tank had its own individual filtration system, but the larval and juvenile grow-out systems were connected in a semi-recirculating system, which included a 30,000 gallon reservoir tank where seawater pumped directly from the ocean was sterilized with chlorine and dechlorinated before use in the system. A header tank, rapid sand filter, biofilters, and protein skimmer completed the filtration system. The larval tanks were located inside the hatchery building and the grow-out tanks and live foods production tanks were located in a greenhouse outside the hatchery. The grow-out period was approximately four months.

Aquaculture Development A/S (ADAS) in Denmark, was also producing clownfish commercially, but is no longer in operation. The company commercially produced ten species of clownfish, using synthetic seawater (Instant Ocean) and recirculating systems. The main species produced were *Amphiprion frenatus*, *A. ocellaris*, *A. sebae*, and *Premnas biaculeatus*. The company began supplying the aquarium markets in Scandinavia, Great Britain, Germany, and the Netherlands with tank-raised anemonefish in 1990, and in November, 1991, the first Danish-produced clownfish were introduced to the American market (Torben, 1992).

The hatchery facility at ADAS consisted of a broodstock system, a larval rearing system, a juvenile grow-out system and a live food production area. The broodstock system contained about 16,000 liters of water that was circulated through sand filters and two UV sterilizers before returning to the 98 spawning tanks. Each 125 liter spawning tank contained an undergravel filter for biological filtration. The larval rearing system contained about 1000 liters of water in four tanks. The grow-out system had a capacity of 52,000 liters and was separate from but similar in design to the broodstock filter system. The live food production system was probably the most sophisticated part of the facility, with two species of microalgae, *Rhodomonas* and *Isochrysis*, grown in two 600 liter photoreactors. Algae production was fully automated, with a maximum daily production of 400 liters of algae. Rotifers were produced in seven tanks, each with a volume of 800 liters. The company developed it own dry food for grow-out of the juvenile fish (Torben, 1992).

Instant Ocean Hatcheries, founded and managed by Frank Hoff, was in operation from 1974 to 1984, producing clownfishes and neon gobies commercially at an inland hatchery in central Florida. Annual production of juveniles reached over 250,000 using Instant Ocean artificial sea salts in a closed culture system. Species produced included: *Amphiprion akallopisos*, *A. clarkii*, *A. ephippium*, *A. frenatus*, *A. ocellaris*, *A. melanopus*, *A. percula*, *A. polymnus*, *Gobiosoma oceanops*, and *Premnas biaculeatus*. In addition, Instant Ocean produced three hybrids: *A. frenatus x A. ephippium*, *A. frenatus x A. melanopus*, and *A. ephippium x A. melanopus*. The hatchery was an 11,200 ft^2 split level building that contained a 10,000 gallon larval rearing system, a 12,000 gallon

broodstock system and a 24,000 gallon grow-out system. The larval rearing system contained 80 forty gallon aquariums. The broodstock system contained 180 plywood tanks of 29 or 40 gallons. The grow-out system contained 384 forty gallon plywood tanks. The aquariums in each system were connected to reservoirs and filters. The facility also contained a 10,000 gallon experimental system used for growing algae, invertebrates and extra pairs of subadult fish. There were ten additional 4,000 gallon reservoirs and six 10,000 gallon outdoor recycling ponds. Approximately 70% of the water used in the hatchery was recycled (Hoff, In Prep).

Some research has been conducted by a few universities, public aquariums, and private research institutions on the culture of marine aquarium species. The University of Hawaii, in a research program under the direction of Dr. Chris Brown, was successful in rearing two species of damselfish, *Dascyllus albisela* and *Dascyllus aruanus*, to the juvenile stage, with one of the rearing trials resulting in over 40% survival. Larval feeding during the first two weeks post-hatch was accomplished by maintaining a continuous indoor culture of algae (*Tetraselmis* sp.) and rotifers in the larval tanks, supplemented by progressively larger wild zooplankton (Danilowicz and Brown, 1992). Dr. Brown is presently working on developing methods for captive spawning of *Centropyge fisheri*, *C. loriculus*, and *C. potteri* (C. Brown, per. comm., 1996).

Harbor Branch Oceanographic Institution (HBOI), Fort Pierce, FL began an ornamental marine fish and invertebrate culture program in 1993. To carry out this research, the Institute equipped a 450 ft^2 glass greenhouse with three independent seawater systems, each containing four 40-gallon aquaria, a wet/dry biofilter and foam fractionator, a GAC contactor, and UV sterilization. The facility also houses translucent fiberglass columns for phytoplankton and marine zooplankton production, and four 3000-liter recirculating larviculture systems. HBOI has successfully reared larvae of the Banded coral shrimp, *Stenopus hispidus*, through metamorphosis after 102 days and the Peppermint shrimp, *Lysmata wurdemanni*, after 35 days. In addition, the Sergeant major, *Abudefduf saxatilis* was successfully cultured. The Institute is also conducting research on the following species:

Species	**Common Name**
Lysmata grabhami	Scarlet lady
Periclimenes pedersoni	Pederson's cleaner shrimp or Caribbean anemone shrimp
P. yucatanicus	Clown anemone shrimp
Stenopus scutellatus	Golden banded coral shrimp

The University of Texas recently began a research program to investigate the culture of marine ornamental fish and shrimp. Under the direction of Dr. Joan Holt, the following species were spawned in the laboratory: *Bodianus pulchellus*, *Centropyge argi*, *Equetus umbrosus*, *Halichoeres caudalis*, *H. maculipinna*, *Lysmata wurdemanni*, *Serranus tigrinus*, and *Thalassoma bifasciatum*. A large tank (i.e., 1000 gallon raceway) was required to get adult *B. pulchellus* to spawn. However, larval rearing has not been successful for any of the fish species, except *B. pulchellus* and *E. umbrosus*, due to a lack

of a suitable first food for the tiny larvae. Rotifers are too large as a first food for these species. Wild plankton was also used without success for some of the fish species. However, the laboratory has been more successful in culturing the Peppermint shrimp *Lysmata wurdemanni* through the larval and juvenile stages. The Peppermint shrimp can be reared through the larval stage in 30 to 50 days, depending on water temperature and other factors. High water quality and the use of enriched *Artemia* are important for successfully culturing this species (J. Holt, per. comm., 1996).

The University of Wales began a research program in 1993 to rear several species of cleaner shrimp. Dr. David Fletcher, principal investigator, has succeeded in rearing *Lysmata amboinensis*, *L. debelius* and *Stenopus hispidus*, with the cultured juveniles now producing larvae in the laboratory (D. Fletcher, per. comm., 1994). He is now developing techniques for the mass culture of the two *Lysmata* species, and refinements in rearing techniques has reduced the larval period for these species from 13 or 14 weeks to seven weeks (D. Fletcher, per. comm., 1996). Of the three species listed above, *S. hispidus* is the most difficult to culture through to the juvenile stage. Rotifers are not required as the first feed for this species, and Dr. Fletcher has had success with a variety of live and inert diets. In particular, a nematode species enriched with marine lipids and pigments was used to supply the essential nutrient profile for these crustaceans (D. Fletcher, per. comm., 1994).

Dr. Fletcher is also investigating nutritional requirements of clownfish, including vitamin C requirements in *Amphiprion clarkii* (Fletcher and Wilson, 1996). He is consulting with a private company to develop new diets for clownfish and other marine aquarium fish. He is also consulting with another private company to develop commercial rearing methods for other species of marine aquarium fish, including tangs. The company has been successful in rearing the Catalina goby, *Lythrypnus dalli*, and may be the first to culture this species through the larval stage. They have also been very successful in rearing a variety of seahorse species, producing a second generation in just 7 to 11 weeks. Improved nutrition has been an important factor in the success with seahorses. This group is also developing new live feeds for species with very small larvae (i.e., larvae that cannot consume rotifers at first-feeding) and new technology for induced breeding (D. Fletcher, per. comm., 1996). Emphasis is also being placed on improving broodstock and larval nutrition to achieve greater success with marine aquarium species that have been difficult or impossible to culture in the past.

Various public aquariums have in the past or are presently conducting research on the culture of marine aquarium species. The Waikiki Aquarium, affiliated with the University of Hawaii, conducted small-scale research on culturing marine aquarium shrimp. Under the direction of Syd Kraul, the Aquarium successfully developed rearing methods for the Harlequin shrimp, *Hymenocera picta*. Using enriched Artemia, the Aquarium was able to rear thousands of juveniles. The Aquarium is not presently involved in research on the culture of marine ornamental fish or shrimp, but Mr. Kraul is planning to resume commercial culture of the Harlequin shrimp later this year (S. Kraul, per. comm., 1996). The Berlin Aquarium has been culturing several species of clownfish and selling them to raise funds for various projects at the Aquarium (C. Brown, per. comm., 1996). The