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Use of formulated diets as replacements for *Artemia* in the rearing of juvenile American lobsters (*Homarus americanus*)

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Abstract

American lobsters, *Homarus americanus*, have been successfully reared in hatchery operations for over a century, yet formulated diets have never been commercially produced. In recent years, commercial *Artemia* replacement (CAR) diets have been developed and marketed for use in aquaculture production of marine shrimp. Three separate experiments assessed the utility of rearing American lobsters on these shrimp CAR diets. First, survival and growth of stage IV American lobsters fed one of three CAR diets (Artemac 5, CAR1; Economac 4, CAR2; and Progression 3, CAR3) were compared to those of animals fed frozen adult n-3 fatty acid enriched *Artemia*. Survival to 3 months was highest for animals fed CAR3 (85%), while animals fed *Artemia* had the greatest weight gain (>6 % day^{-1}). A cost/benefit ratio analysis showed that CAR2 was the most cost efficient for juvenile production because of its low overall purchase cost. Second, stage IV lobsters were fed either CAR2 or frozen adult n-3 fatty acid enriched *Artemia* exclusively, or in combination (2:5, and 5:2). Again, CAR2 was a cost effective feed to use, even as a partial replacement for *Artemia*. Survival was higher in diets that included CAR2, and feeding it two days per week compensated for low quality *Artemia*. Finally, 1.5 year old lobsters fed a gelatin-bound mix of 80% CAR2 and 20% frozen *Artemia* for five months survived and grew equally well compared to lobsters fed gelatin-bound frozen adult *Artemia*, and better than a custom formulated maintenance diet. The benefits of incorporating formulated feeds into American lobster rearing programs to increase the effectiveness of enhancement programs is discussed.
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1. Introduction

Formulated, commercially available feeds are preferred for use in aquaculture operations due to their lower cost, ease of storage, and reduced incidence of bacterial contamination compared to live or frozen foods (Cox and Johnston, 2003; Fiore and Tlusty, 2005). For early juvenile American lobsters (*Homarus*

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americanus), live adult *Artemia* is considered to provide optimal nutrition (Conklin, 1995), although the cost of its purchase or production is prohibitive for large-scale hatchery use. The traditional alternative has been to feed frozen adult *Artemia,* which supports growth rates approximately 60% of that of live *Artemia* (Conklin, 1995). Research on early benthic juveniles in the 1970s and 1980s determined that protein and lipids were the most critical components for maximal survival and growth. Minimum dietary protein level for rapid growth was determined to be approximately 40%, optimal crude lipid was 14–15%, and cholesterol and phospholipids were found to be essential in preventing molt death syndrome (Conklin, 1995; D'Abramo et al., 1981). Excellent formulated diets (high survival and growth rates 60% to 80% that of the live *Artemia* standard) were developed in the lab (Conklin et al., 1980; D'Abramo and Conklin, 1985; Bordner et al., 1986). Unfortunately American lobster aquaculture efforts were largely abandoned by the early 1990s due to technological gaps in rearing management (Aiken and Waddy, 1995) coupled with a strong resurgence in fishery landings (National Marine Fisheries Service, 2002). Consequently, the absence of an industry precluded formulated juvenile lobster diets from becoming commercially available (Aiken and Waddy, 1995).

Interest in the aquaculture production of American lobsters is now growing because of concerns of overfishing, health issues, and a rapid decline in lobster stocks south of Cape Cod, Massachusetts, particularly in the Long Island Sound fishery (Atlantic States Marine Fisheries Commission, 2004). In addition, European lobster (*Homarus gammarus*) stocks have been successfully enhanced. When stocks in Norway declined to 30% of historic levels, a concerted effort was directed to augment the fishery. The Norwegian program reared juveniles for 5 to 19.5 months (to 11.8–21.1 mm carapace length) in the hatchery before release, and was ultimately successful with 50% of fishery landings being of hatchery origin (Agnalt et al., 1999). Similarly, in the United Kingdom, wild stocks were increased through the release of 15 mm CL (stage X to XII) lobsters (Addison and Bannister, 1994). These scenarios therefore offer promise that an American lobster enhancement program could succeed through the adoption of European methodology.

At present, the augmentation of American lobster stocks needs to be re-evaluated based on (1) severe and widespread stock declines, and (2) the potential feasibility of homarid lobster enhancement based on the European model. In addition to a probable need and the potential for success, the past two decades have brought rapid increases in global aquaculture production. This success stems from improvements in the very areas in which American lobster aquaculture was lacking including hatchery technology, broodstock management, and in particular, developments in feed production (Aiken and Waddy, 1995). Of these, the advancement of the feed industry has been most significant, particularly in the crustacean sector. Artificial diets are now utilized in the production of all life history stages of marine shrimp, including commercial *Artemia* replacement (CAR) diets manufactured for postlarval penaeid shrimp. In both penaeid shrimp and clawed lobster postlarval stages, *Artemia* has been shown to provide excellent nutrition (Wickins and Lee, 2002; Conklin, 1995). As CAR diets developed for shrimp have been formulated to match *Artemia* nutritionally, they may also be an effective and economical off-the-shelf feed for the hatchery production of early benthic phase juvenile lobsters.

Previous work has shown that some CAR diets can be used as partial replacements for live or frozen *Artemia* in the rearing of pelagic lobster larvae (Fiore and Tlusty, 2005). Here, three experiments were conducted to determine whether these CAR diets are a viable alternative to frozen *Artemia* as a hatchery diet for juvenile American lobsters. Temperature and feeding schedules in our facility had been optimized for production and long-term maintenance of juveniles rather than for promoting maximum growth rates. Therefore, these experiments were designed to evaluate performance on formulated diets relative to the frozen *Artemia* standard. The First experiment examined weight gain, condition, and survival of early juvenile lobsters beginning at stage IV fed one of three different CAR diets, or a frozen n-3 fatty acid enriched adult *Artemia* diet. The Second experiment examined the performance of early juveniles beginning at stage IV fed combinations of the best performing CAR diet and frozen n-3 fatty acid enriched adult *Artemia*. To determine the most cost effective diets within these two experiments, the survival and biomass benefits were compared with

feed costs and analyzed graphically. Finally, in the Third experiment, 18-month-old lobsters were grown for five months on gelatin-bound forms of the best performing CAR diet, frozen *Artemia*, or a maintenance diet to assess whether the best performing CAR diet was suitable for older juvenile lobsters.

2. Materials and methods

2.1. Facility and animals

Three feeding experiments were conducted in a small research hatchery at the New England Aquarium, Boston, MA USA. Animals were maintained in a semi-closed flow-through filtered sea water system (salinity 30.5–35.0‰, pH 7.84–7.97, NH_4 <70 mg l^{-1}) and were subjected to a daily artificial light cycle (13 : 11 L / D, full-spectrum fluorescent lighting). All experimental animals were cultured in the hatchery, and hatched from ovigerous American lobsters that were wild-caught along the southern coast of Massachusetts. Upon hatching, larvae were reared in 40 l Hughes planktonic kreisels (Hughes et al., 1974) at 15–20 °C (average. 16.86 °C) and fed Super-Selco® enriched *Artemia* (Salt Creek Inc., South Salt Lake City, UT) nauplii and frozen adult n-3 enriched *Artemia* (San Francisco Bay Brand, San Francisco, CA). Upon metamorphosis to stage IV,

postlarvae were transferred to individual 33 mm diameter rearing cups, placed in a flow-through fiberglass seatray, and fed *Artemia* nauplii and frozen adult *Artemia*. Lobsters for experiments One and Two were held under these conditions for two to six days at which point postlarvae were then randomly divided into their treatment groups while ensuring that animals were matched across all treatments for hatching and metamorphosis dates. Lobsters for Experiment 3 were held under these conditions at a mean temperature of 18.5 °C for five months from hatching, after which time they were transferred to a second flow-through seatray system where they were fed a diet of frozen adult n-3 enriched *Artemia* and held at a mean temperature of 12.4 °C in individual 15.9 cm^2 plastic mesh containers (RN 2221, InterNet Inc, Minneapolis, MN). Finally, four months before the beginning of Experiment 3, lobsters were transferred into individual 70.9 cm^2 plastic mesh containers (RN 3531, InterNet Inc, Minneapolis, MN), while still being maintained on frozen adult n-3 enriched *Artemia* at approximately 12 °C.

Water temperature during the periods of all experiments averaged 16.9 °C, (min. 15 °C, max. 22 °C).

2.2. CAR diets

Three suitable CAR diets were evaluated in this experiment: Artemac 5 (CAR1), Economac 4 (CAR2),

Table 1
Feed composition and source for diets used throughout the juvenile feeding experiments

Diet	Frozen *Artemia*[a]	CAR1	CAR2	CAR3
		Artemac 5[b]	Economac 4[b]	Progression 3[c,d]
Size	Adult	500–800 μm	500–800 μm	100–250 μm
Type	*A. salina* Omega-3 enriched	Micro-encapsulated	Micro-particulate	Micro-encapsulated
Cost (as sold)	$18/kg	$37.50/kg	$9.50/kg	$38/kg
Cost/kg as fed (dry wt.)	$140 (estimate)	$37.50	$9.50	$38
Proximate analysis[e]				
Crude protein	5.02% Min.	57.0%	57.0%	50% Min.
Crude fat/ total lipid	1.3% Min.	19.0%	13.5%	12% Min.
Carbohydrate (by subtraction)	–	12.0%	15.7%	–
Fiber	0.29% Max.	–	–	–
Ash	–	5.0%	7.0%	12% Max.
Moisture	92.5% Max.	7.0%	6.9%	5% Max.

[a] San Francisco Bay Brand, Inc. 8239 Enterprise Dr., Newark, California 94560.
[b] Aquafauna Bio-Marine, P.O. Box 5, Hawthorn, CA 90250.
[c] Salt Creek Inc, 3528 West 500 South Salt Lake City, UT 84104.
[d] Diet was provided for free by manufacturer in exchange for a copy of the experimental results.
[e] All values are as supplied by vendors.

and Progression 3 (CAR3, Table 1). These diets were selected due to their similarity to the nutritional profile of a reference diet established for lobsters (Conklin, 1995) or the nutritional profiles of adult *Artemia*. All CAR diets were purchased prior to the experiments. Three lots of frozen adult *Artemia* were purchased (San Francisco Bay Brand, San Francisco, CA), one for each experiment.

2.3. Experiment 1 (CAR diet comparisons)

The first experiment was a direct comparison of the performance of juveniles fed one of three CAR diets to that of juveniles fed a frozen adult n-3 fatty acid enriched *Artemia* diet (control, referred to as B). Frozen *Artemia* was selected as a comparison diet as it is readily available, has been previously used as a reference diet (Conklin, 1995) and is presently used as the standard diet in *Homarus* spp aquaculture operations (Kristiansen et al., 2004). Sibling stage IV postlarvae were assigned to one of the four test diets ($n = 40$ per treatment), assigned a unique identification number, weighed, and placed in individual rearing cups (InterNet Inc, Minneapolis, MN, RN2630 RC tube with Internet Inc XN3019 mesh bottom). The sinking nature of CAR2 necessitated the use of a finer mesh (Internet Inc #XN 7110) on the bottom of all rearing cups to contain this feed. To eliminate diet cross contami-

nation, juvenile rearing cups were grouped by treatment in separate flow-through tubs (Fig. 1). Flow-through tubs were adjacent to each other in one seatray and utilized the same water source. Lobsters were fed their test diets in excess each morning, and allowed to feed for approximately 4–6 h before all remaining food was siphoned from each cup and tub. Thus, all lobsters had equal access to feed for a limited amount of time per day. Additionally, lobsters were permitted to consume their molted exoskeletons.

2.4. Experiment 2 (CAR2/Artemia mixed diet evaluation)

A second experiment was conducted to evaluate the efficacy of CAR2 as a total or partial replacement diet for frozen n-3 fatty acid enriched *Artemia*. Four dietary treatments included animals fed: frozen n-3 fatty acid enriched adult *Artemia* only (0/7), CAR2 only (7/7), CAR2 five days per week and frozen *Artemia* two days per week (5/7), and CAR2 two days per week and frozen *Artemia* five days per week (2/7). Sibling stage IV postlarvae were randomly divided among each dietary treatment ($n = 40$ per treatment). Animals were fed in excess for 4 to 6 h daily, and the experimental system and rearing protocol were the same as in Experiment 1 (Fig. 1).
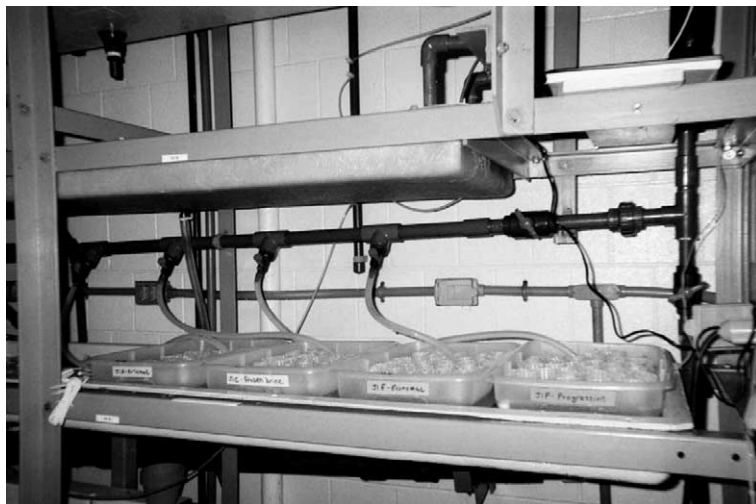

Fig. 1. The experimental setup for experiments One and Two. All rearing cups for a single diet are placed within a flow-through tub in a flow-through seatray. Water depth was approximately 5 cm.

2.5. Experiment 3 (18-month juveniles)

Eighteen month old lobsters (15.17 ± 0.953 mm CL, average ± 1 S.D.) that had been reared on a diet of frozen n-3 fatty acid enriched adult *Artemia* were randomly divided among three groups of 29–30 animals each. Seventy-eight of these lobsters were siblings, and were allocated equally and randomly among dietary treatments. Each group was fed one of three gelatin-bound diets: (B) frozen n-3 fatty acid enriched adult *Artemia* bound in gelatin (68.2% and 31.8% respectively dry weight basis), (E) 90.5% CAR2 , 4.5% frozen adult *Artemia*, bound in 5.0% gelatin; and (G) a mix of 4.6% frozen adult *Artemia*, 19.9% krill meal, 19.9% fish meal, 15.9% soy lecithin, 7.9% kelp meal, 6.3% spirulina, 20.5% vitamin/mineral/bone meal mix, 0.01% astaxanthin, bound by 5.0% gelatin. Fresh lots of all dietary ingredients were purchased prior to this experiment (G diet dry ingredients from Aquaculture Supply, Dade City, FL). G diet is a 35% protein maintenance diet developed at the New England Aquarium where it has been used to maintain 1+ year old lobsters, with sufficient survival, growth, and molt condition (Tlusty and Goldstein, unpublished data). Lobsters were held individually in 70.9 cm^2 plastic mesh containers placed directly in a flow-through fiberglass seatray (water depth approximately 10 cm, salinity 35‰, 15–19 °C). In all diet treatments, lobsters were fed to excess in the morning three days per week, and wastes and uneaten food were siphoned out approximately 6 h later. Lobsters were permitted to consume their molted exoskeletons. The presence of molts, mortality and any physical or behavioral abnormalities were recorded daily.

Hemolymph samples were collected from each juvenile lobster on day 0 prior to being fed the experimental diets. A standard 1 cc insulin syringe (28-gauge needle) was used to draw a 0.05 ml hemolymph sample from the sinus of the 5th walking leg. Five microliters of this sample was placed on a hand-held refractometer (Leica TS Meter Refractometer, Leica Microsystems Co.) to measure blood refractive index (RI). The remaining sample was centrifuged in a microcentrifuge, 10 μl of the supernatant was placed onto a Lifescan® One Touch blood glucose test strip, and glucose values were read directly from a Lifescan® Profile glucometer (Life-

Scan, Inc., Milpitas, CA USA). Molt stage, which can significantly influence blood chemistry including glucose and protein, was determined at blood sampling periods by removing the tip of a pleopod (swimmeret) using sharp fine scissors, and staged according to Aiken (1973). Molt stages include the postmolt stages of A, B and C1 to C3, intermolt (C4), and the premolt stages of D0 to D3 (Mercaldo-Allen, 1991). Carapace length was measured to the nearest 0.1 mm with vernier calipers and weight was determined using a Mettler AB50 balance. The 150 day weight and length samples were converted to Δ% (from day 0) and then analyzed as such. Subsequently, a condition factor (CF) was calculated as: Weight (g)/CL (mm)^3 * 100.

Unfortunately, the volume of hemolymph necessary for glucose testing was excessive for the size of the lobster at the beginning of the experiment, and significant mortality was observed during the seven days following the drawing of the initial sample of hemolymph. Thereafter, hemolymph was only sampled for RI at 30 days, and nine animals (three from each treatment) were tested for glucose and RI on day 150. In addition to the difficulty in securing an adequate hemolymph sample, other lobsters held in the recirculating system appeared to develop a bacterial infection on approximately day 45. As a precautionary measure, all animals were given a 5 day treatment of oxytetracycline at that time (19.2 μg oxytetracycline/g wet weight lobster).

At the termination of the experiment on day 150, three lobsters from each treatment were sacrificed and sent to the Arizona Veterinary Diagnostic Laboratory (University of Arizona, Tucson, AZ) for pathological testing. Mid-gut gland samples were scored for lipid content on a 0 (absent) to 4 (abundant) scale (D. Lightner, pers. comm.), carapace thickness was measured from H&E stained slides from a standardized location, and animals were assessed for superficial fouling.

2.6. Statistical analyses

In all experiments, molting, mortalities and abnormalities were recorded daily. At one month and at three months after the beginning of experiments One and Two, wet weights were recorded. Percent growth per day was calculated as the percent increase in

weight between the initial measurement at postlarval stage IV and the one or three month measurements. At the three month sample, percent growth per day, specific growth rate (SGR, $(\ln(\text{weight}_{\text{final}}) - \ln(\text{weight}_{\text{initial}})*100)/\text{number of days})$, and normalized biomass index (NBI, Conklin et al., 1975, ((total wet weight$_{\text{final}}$ − total wet weight$_{\text{initial}}$)/initial number of lobsters)), was also determined. In Experiment 3, percent increases in lobster lengths and weights were determined only for the entire 150-day period, rather than for individual monthly determinations. Of the nine lobsters sampled for hemolymph RI and glucose at 150 days, eight were at molt stage C. One lobster fed diet E diet was at molt stage A/B and was omitted from statistical analyses, as molt stage influences hemolymph chemistry (Mercaldo-Allen, 1991). Normal data were analyzed without transformation, while non-normal data were first ranked, and then analyzed using a one or two way (Kruskal–Wallis) ANOVA (Zar, 1984). Paired comparisons were made using Tukey's (normal data) or Dunn's (non-normal data) tests.

Survival data for all three experiments were analyzed using a log–rank Kaplan–Meier survival analysis with multiple comparisons performed with the Holm–Sidak method (SigmaStat 3.0, Systat, Richmond, CA).

2.7. Cost/benefit analyses

To determine the relative value of each of the different diets used in experiments One and Two, benefits and costs were graphically analyzed. Relative benefits were calculated for both the number of animals being produced, and the biomass of animals. Both benefits and costs were first set relative to the frozen *Artemia* diet (B in Experiment 1, 0/7 in Experiment 2). This diet was used as the reference diet because of its historical use in the hatchery production of juvenile lobsters (Conklin, 1995). Since the *Artemia* diet was the reference diet, relative benefits equaled costs and the B:C ratio was considered as 1.0. The relative benefit and cost of each diet was then plotted and compared to the reference diet. A benefit:cost ratio reference line equal to 1.0 was also plotted, and the ratio of those experimental diets that was above the line was deemed as most cost-effective for production of juvenile lobsters.

The per kilogram cost breakdown (dry weight, excluding labor, shipping and taxes) for each of the diets used in Experiment 1 at the time of their purchase from commercial retailers was as follows (see also Table 1): Progression 3 $38/kg$_{\text{dw}}$, Artemac 5 $37.50/kg$_{\text{dw}}$, Economac 4 $9.50/kg$_{\text{dw}}$, and frozen n-3 enriched adult Artemia was $140/kg$_{\text{dw.}}$ The cost/kg$_{\text{dw}}$ for the diets used in Experiment 2 were based on the proportional use of Economac 4 and *Artemia* and respective costs were 0/7=$140, 2/7=$103, 5/7=$47, and 7/7=$9.50.

3. Results

3.1. Experiment 1 (CAR diet comparisons)

For the first two to three days, juveniles were observed feeding on all of the diets. For the following four days, no feeding was observed except in the frozen *Artemia* group. After this period of inactivity, juveniles resumed feeding on all diets and no other feeding interruptions were observed.

Diet had a significant effect on the survivorship of the juvenile lobsters beginning at stage IV (Kaplan–Meier Survival Analysis, $df=9$, $S=9.04$, $P<0.03$). Animals fed the CAR3 diet had the highest survivorship (85%), while those fed the CAR1 had the lowest survivorship (60%, Holm–Sidak method=9.21, $P<0.005$, Table 2). All other multiple comparisons showed no statistical differences (Holm–Sidak method <6.00, $P>0.05$).

In general growth rates, measured as percent weight gain per day, associated with the commercially manufactured diets were not as great as those with the frozen *Artemia* diet. After four weeks, animals fed the *Artemia* diet showed a significantly greater growth rate than any CAR diet (Kruskal–Wallis one way ANOVA, $df=3$, $H=105.19$, $P<0.001$, Dunn's test, $Q>2.91$, $P<0.05$ for all comparisons, Table 2). Among the CAR diets, growth of juveniles fed CAR2 was greater than that of animals fed either CAR1 (Dunn's test, $Q=5.51$, $P<0.05$) or CAR3 (Dunn's test, $Q=5.55$, $P<0.05$). A similar trend was evident after three months, except growth for juveniles fed either the *Artemia* or the CAR2 diet was not statistically different (Kruskal–Wallis one way ANOVA, $df=3$, $H=81.74$, $P<0.001$, Dunn's test, $Q=2.57$,

Table 2
The mean percent growth per day and survival (cumulative %) one and three months after diet experiments commenced for juvenile lobsters in Experiment 1 fed either frozen *Artemia* or one of three commercially formulated diets, and in Experiment 2 fed frozen adult *Artemia* only (0/7), CAR2 only (7/7), CAR2 five days per week and frozen *Artemia* two days per week (5/7), or CAR2 two days per week and frozen *Artemia* five days per week (2/7). For 3-month data, final weights, specific growth rate (SGR) and Normalized Biomass Index (NBI) are also calculated. Within experiments statistical similarity is denoted by similar superscripts. Values are averages ± 95% C.I.

Diet	1 month		3 months				
	Percent growth d^{-1}	Survival (%)	Percent growth d^{-1}	Survival (%)	Final wt. (mg)	SGR	NBI
Experiment 1							
Artemia	2.76 ± 0.43[a]	90.0	6.29 ± 0.58[a]	67.5	292.96 ± 19.3[a]	2.10 ± 0.08[a]	152.90
CAR2	1.87 ± 0.12[b]	90.0	3.49 ± 0.57[a]	65.0	187.28 ± 21.4[a]	1.54 ± 0.14[a]	68.89
CAR3	0.83 ± 0.14[c]	90.0	1.68 ± 0.17[b]	85.0	110.45 ± 8.2[b]	1.01 ± 0.07[b]	19.46
CAR1	0.84 ± 0.12[c]	77.5	1.18 ± 0.14[b]	60.0	88.38 ± 5.3[b]	0.79 ± 0.07[b]	29.83
Experiment 2							
0/7	2.65 ± 0.14[a]	55.0	5.96 ± 0.78[a,b]	22.5	303.48 ± 30.8[a]	2.05 ± 0.13[a,b]	85.29
2/7	2.50 ± 0.11[a]	75.0	5.88 ± 0.53[a]	67.5	307.19 ± 22.9[a]	2.03 ± 0.09[b]	158.49
5/7	2.13 ± 0.17[b]	67.5	4.30 ± 0.92[b,c]	65.0	235.54 ± 29.2[b]	1.73 ± 0.18[b,c]	70.71
7/7	1.71 ± 0.14[c]	67.5	3.42 ± 0.56[c]	62.5	194.01 ± 25.0[b]	1.52 ± 0.13[c]	68.19

$P > 0.05$, Table 2). Lobsters fed the *Artemia* diet reached approximately 300 mg while those fed the CAR1 and CAR3 diets were approximately 100 mg (Table 2). Along with greater weight gains, lobsters fed the frozen *Artemia* diet were at a more advanced stage at 3 months than that of those fed the commercially manufactured diets ($\chi^2 = 117.20$, $df = 12$, $P < 0.001$). The modal stage for juveniles fed the frozen *Artemia* was VIII, CAR1 and CAR2 animals were at VII, while the animals fed CAR3 were at stage VI. In all cases, modal values were >60%. Higher growth rates and the average survivorship for juveniles fed the frozen *Artemia* diet led to this treatment having the greatest biomass at both one and three months. Feeding CAR2 resulted in a total biomass that was 85% (one month) and 60% (three months) of that realized for lobsters fed frozen adult *Artemia*. CAR1 and CAR3 diets produced biomass levels of approximately 60% that of frozen *Artemia* at 4 weeks, and <40% of that at three months. The corresponding NBI values were 153 for the *Artemia*-fed lobsters, 69 for CAR2, and <50 for CAR diets 1 and 3 (Table 2).

While lobster fed the frozen *Artemia* diet exhibited the greatest gain in biomass, they also exhibited the highest number of molt related problems. Animals in this treatment exhibited the greatest prevalence of missing appendages associated with molt related difficulties. Approximately 80% of the animals in this treatment were missing at least one appendage after one month, and 55% after three months. By comparison, for the CAR diets, 41.2% of CAR2-fed, 11.7% of CAR3-fed, and 61.3% of CAR1-fed lobsters exhibited molt-associated appendage loss after one month. These values declined to 20%, 6.3%, and 4.3% respectively by the third month.

After one month, diet-dependent differences in lobster cuticle color were evident. *Artemia*-fed lobsters remained the most similar to wild coloration indicated by a dark mid-gut gland and cuticle pigmentation. Lobsters fed the CAR diets were paler with CAR2-fed lobsters being the palest. Color differences became more pronounced so that by the end of the experiment, the cuticles of CAR2-fed juveniles appeared colorless, and they exhibited a very pale orange mid-gut gland. At the conclusion of the experiment, stage VIII CAR2-fed juveniles were switched to a diet of frozen *Artemia* supplemented with live *Artemia* nauplii, acquiring normal wild-type coloration in just over two weeks.

3.1.1. Cost/benefit

Significant differences in the relative benefits and costs of each dietary treatment used in this experiment were observed (Fig. 2). Formulated diets were significantly less expensive than the frozen *Artemia* diet. As a result of this cost differential, the benefit:cost ratios for both biomass and number of survivors for
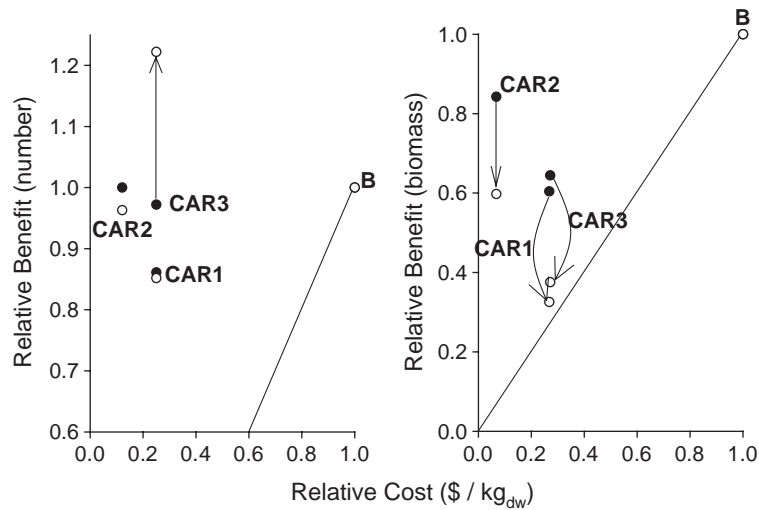
Fig. 2. The relative benefit (left—number, right—biomass) compared to the relative costs for each diet fed to juvenile lobsters at one (closed circles) and three months (open circles). Arrows indicate shifts in benefits between the two sample intervals. Frozen *Artemia* (B) was used as the reference diet. The reference line indicates B/C ratios of 1 : 1. CAR diets are identified as in Table 1.

each of the three formulated diets were greater than that of frozen *Artemia* (they were above the 1 : 1 reference line, Fig. 2). As lobsters grew from one month to three months (right graph, Fig. 2), B : C ratios for biomass of the CAR diets approached the reference line, but were still greater than 1.0. In addition, biomass values were closer to the reference line than the corresponding values for numbers. In all cases, the slope of the line from the origin to the data point was highest for animals fed CAR2 indicating this diet had the highest B : C ratio.

3.2. Experiment 2 (CAR2/Artemia mixed diet evaluation)

Survival in this experiment was significantly impacted by diet (Kaplan–Meier survival analysis $=20.471$, $df=3$, $P<0.001$). Survival of juveniles fed frozen *Artemia* exclusively (0/7) was significantly lower than that of any other treatment (Holm–Sidak multiple comparison method >8.54, $P<0.005$, Table 2) and was significantly less than the 67.5% survival observed in the previous experiment (Table 2). Lobsters fed only CAR2 in this experiment demonstrated survivorship (72.5%, Table 2) that was slightly greater than that achieved in the previous experiment (65%, Table 2). Survivorship between the pure CAR2 (7/7) and mixed CAR2/

Artemia diets (2/7 or 5/7) did not differ significantly (Holm–Sidak multiple comparison method <0.738, $P>0.30$, Table 2).

While CAR2 diets yielded better survivorship than that achieved with the frozen *Artemia* exclusively (0/7) diet, the growth of animals demonstrated a reverse trend. The percent growth per day varied with dietary treatment (one way ANOVA, one month data $F_{3,86}=34.50$, $P<0.001$, three month data $F_{3,68}=15.10$, $P<0.001$, Table 2), and was lowest for the pure CAR2 diet (7/7). At four weeks, all paired comparisons to 7/7 were statistically different (Tukey's test, $q>4.89$, $P<0.005$), while at three months 7/7 was significantly less than 0/7 (Tukey's test, $q=6.44$, $P<0.001$) and 2/7 (Tukey's test, $q=8.69$, $P<0.001$) but not 5/7 (Tukey's test, $q=2.49$, $P>0.25$, Table 2). While the 0/7 diet had the greatest growth, it was not significantly different from the 2/7 diet (Tukey's test, one month, $q=2.10$, $P>0.40$; three months $q=0.22$, $P>0.95$), and the 5/7 diet at three months (Tukey's test, $q=3.70$, $P>0.05$, Table 2). Lobsters fed the 0/7 and 2/7 diets exceeded 300 mg by the end of the experiment, while the lobsters fed the 5/7 and 7/7 diets were approximately 200 mg (Table 2). Differences in survivorship and growth resulted in the 2/7 diet producing the greatest NBI value (158.5), while all other diets had a NBI of half that value (Table 2).

3.2.1. Cost/benefit

Significant differences in the relative benefits and costs of each dietary treatment used in this experiment were observed (Fig. 3). The cost differential resulted in the B:C ratios for both number and biomass for each of the three diets containing CAR2 being greater than that of frozen *Artemia* (they were above the 1:1 reference line, Fig. 3). Relative benefit values for the three CAR2 containing diets increased from one month to the three month sampling periods due to poor survivorship of juveniles fed the 0/7 diet. The most cost effective treatment was the 7/7 diet, as indicated by the greatest slope of the line connecting this treatment to the origin.

3.3. Experiment 3 (18-month juveniles)

Diet significantly influenced survival in the 18-month old animals (Kaplan–Meier survival analysis $=8.365$, $df=2$, $P<0.02$). Lobsters fed all three diets exhibited marked mortality during the first seven days, most likely in response to excessive hemolymph removal. After these first seven days, mortalities decreased, and animals fed the E diet exhibited 90% survival from day 8 through day 150 (Fig. 4). Animals fed the other two diets exhibited reduced survival compared to the E group, although only the

37.5% survival of the G group was significantly lower than that observed for the E group (Holm–Sidak multiple comparison method $=7.22$, $P<0.01$, Fig. 4). Survival of animals fed diet B was intermediate to, and not significantly different from either the E or G groups (70%, Holm–Sidak multiple comparison method <4.95, $P>0.05$, Fig. 4). A slight mortality pulse occurred at 40 to 45 days, at the period of general health problems in the colony. Yet even with this pulse, approximately 40% of the mortality of juveniles in the G group occurred after day 80.

While lobsters fed the B diet had intermediate survival, their percent increase in carapace length was significantly greater than that of either animals fed E or G (one way ANOVA, $F_{2,38}=6.25$, $P<0.005$, Tukey's test, $q>4.0$, $P<0.02$). The length of animals fed the B diet increased approximately 25% over the duration of the experiment (Table 3), and was unlikely a result of a differential number of molts. For surviving animals, no diet-dependent difference in the number of molts per individual was observed (Kruskal–Wallis one way ANOVA, $H=1.73$, $P>0.40$). Animals fed the B diet also demonstrated the greatest weight gain (one way ANOVA, $F_{2,38}=4.61$, $P<0.02$, power $\alpha_{.05}=0.631$) but this was not significantly greater than in the E group (Tukey's test, $q=2.78$, $P=0.10$, Table 3). The results for condition factor
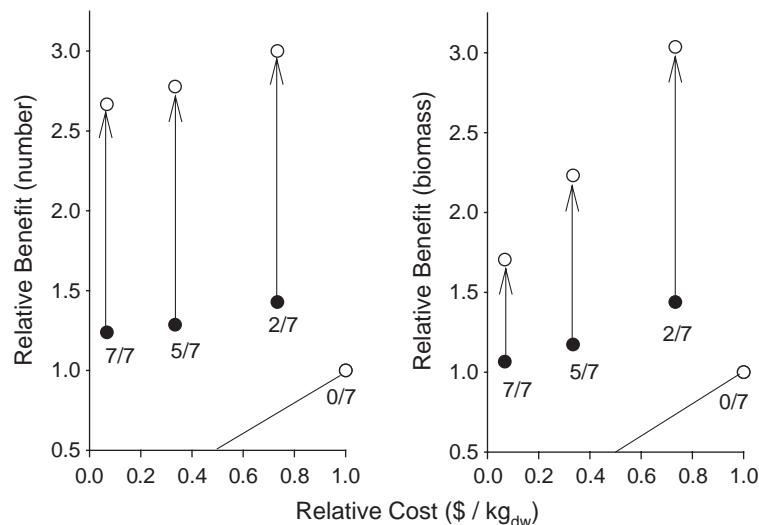

Fig. 3. The relative benefit (left—number, right—biomass) compared to the relative costs of each diet fed to juvenile lobsters for one (closed circles) and three months (open circles). Arrows indicate shifts in benefits between the two sample intervals. Frozen *Artemia* (0/7) is used as the reference diet. The reference line indicates B/C ratios of 1:1. Dietary treatments are identified as in Table 2.
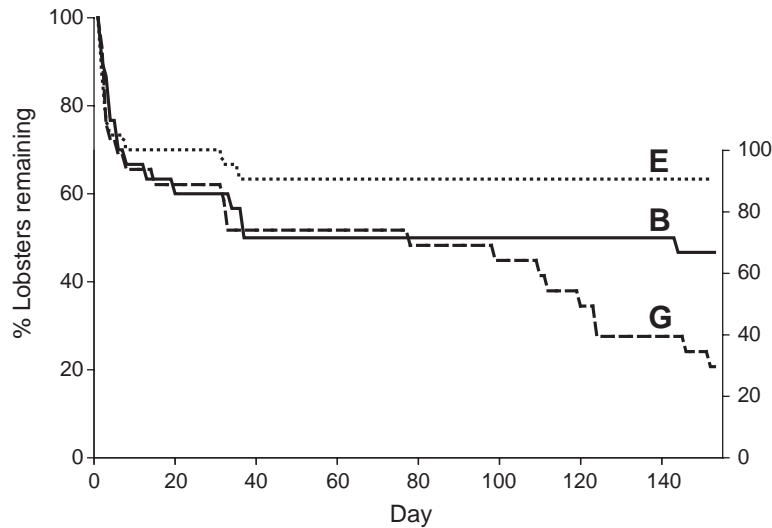
Fig. 4. Survival of 18-month old American lobsters fed one of three experimental diets for 150 days when fed CAR2 based diet (E), frozen adult *Artemia* (B), or a specially formulated diet (G). The left vertical axis represents percent survival for the entire experimental period, while the right vertical axis depicts percent survival after the seventh day of the experiment.

were similar to those for weight, and animals fed the E and B diets had equivalent condition factors (one way ANOVA, $F_{2,38}=5.41$, $P<0.01$, power $\alpha_{.05}=0.729$, Tukey's test, $q=2.72$, $P=0.14$, Table 3).

Hemolymph refractive index (RI) did not vary significantly with diet treatment at the 30 day sample period (Kruskal–Wallis one way Analysis of Variance on Ranks, $H=1.59$, $P>0.40$). The average RI at 30 days, 99.3 ± 4.13 (95% C.I.) was greater than that of the initial measure (75.5 ± 4.31, Kruskal–Wallis one way ANOVA, $H=49.51$, $P<0.001$). However, the difference was likely due to the influence of molt stage on blood chemistry (Kruskal–Wallis one way ANOVA, $H=42.27$, $P<0.001$). The RI of molt stages C4 and

D0 were equivalent, while D1 was equivalent to D3 (no D2 were observed, Dunn's Method, $Q>5.0$, $P<0.05$). At day 0, 91% of animals were at intermolt (molt stage C4 or D0), while at 30 days, this percentage decreased to 57% as more lobsters were at stage D1. At 150 days, significant differences in RI relative to dietary treatment were present for the eight animals at molt stage C (one way ANOVA, $F_{2,7}=13.78$, $P<0.01$, power $\alpha_{.05}=0.91$). Lobsters in the E group had the largest RI (143.5 ± 22.5), which was significantly larger than that for lobsters in the G group (95.67 ± 6.23, Tukey's test, $q=7.34$, $P<0.01$). The RI of lobsters in the B group (120.67 ± 10.87) was intermediate to and not significantly different from the other two diet groups.

Table 3
Initial (day 0) and final (day 150) carapace lengths (CL, mm) weights (WT, g), and condition factor (CF, g mm^{-3}) for juvenile American lobsters in Experiment 3 fed one of three experimental diets. The percent change in carapace length and weight is also calculated. Similar letters denote statistical similarity for each response variable within measurement period

Day	Diet	CL	% Δ CL	WT	% Δ WT	CF
0	B	14.85 ± 0.43		2.18 ± 0.18		0.066 ± 0.002
	E	15.43 ± 0.30		2.46 ± 0.14		0.067 ± 0.002
	G	15.22 ± 0.24		2.33 ± 0.13		0.066 ± 0.002
150	B	19.06 ± 0.73	26.92 ± 3.75[a]	4.79 ± 0.57[a]	106.55 ± 14.84[a]	0.068[a,b] ± 0.002
	E	18.50 ± 0.60	19.43 ± 3.29[b]	4.55 ± 0.46[a,b]	83.65 ± 17.00[a,b]	0.071[a] ± 0.001
	G	18.00 ± 0.37	18.18 ± 3.00[b]	3.92 ± 0.23[b]	63.12 ± 19.42[b]	0.067[b] ± 0.004

At day 0, molt stage did not affect glucose (one way ANOVA, $F_{3,71} = 1.45$, $P > 0.20$), although the preponderance of molt stage C4 and D0 animals may have precluded distinguishing differences at the later molt stages. Glucose levels for the eight animals at stage C did differ significantly among dietary treatments at 150 days (one way ANOVA, $F_{2,7} = 16.93$, $P < 0.01$, power $\alpha_{.05} = 0.96$), with E-fed lobsters (16.5 ± 2.9) having significantly greater glucose values than G-fed (4.7 ± 1.7) and B-fed lobsters (8.3 ± 1.7) Tukey's test, $q > 4.8$, $P < 0.05$). Glucose values for G and B-fed lobsters were not statistically different from one another.

Diet also significantly influenced the energy stores of the mid-gut gland (one way ANOVA, $F_{2,8} = 13.0$, $P < 0.001$, power $\alpha_{.05} = 0.926$). Lobsters fed the G diet had a lower mid-gut gland lipid score than animals fed the B or E diets (Tukey's test, $q > 5.0$, $P < 0.05$). G animals also tended to have a thinner carapace, although this trend was not significant (Kruskal–Wallis one way Analysis of Variance on Ranks, $H = 5.12$, $P > 0.07$). While animals fed the G diet tended to have thinner carapaces, a lesion (shell disease) was observed in one animal fed the B diet. Lobsters fed the E diet exhibited no surface fouling by filamentous bacteria or loricate protozoan, while 1/3 of diet B fed and 2/3 of diet G fed lobsters were observed to have such fouling. The lobsters fed diet G were the only ones to present hemocytic enteritis of the midgut, as two of the three animals were scored as such.

4. Discussion

In this study, moderate growth, excellent survival, and adequate health of lobsters in both the nutritionally sensitive early benthic stages and in juveniles up to two years old was achieved using CAR diets. While relative growth and biomass benefits of lobsters fed CAR diets were in some cases less than that of lobsters fed traditional frozen *Artemia* diets exclusively, it was more economically efficient to use CAR diets because of their low purchase cost relative to that of the *Artemia*. This study was unique in identifying an off-the-shelf commercially formulated diet that could be used today for early juvenile production, at a lower cost than with the feeds currently being used

for the early juveniles in European hatcheries (Kristiansen et al., 2004).

Among the CAR diets, notable differences in lobster growth and survivorship were observed. Survival of animals fed only CAR2 was among the greatest of any diet in the first two experiments. The CAR2 diet resulted in the best growth rate of the three formulated diets tested, although growth was still less than that with frozen *Artemia*. When this formulated diet was fed in combination with frozen *Artemia* (2/7 or 5/7 in experiment Two), growth significantly improved, with the growth of lobsters fed the 2/7 diet being equivalent to that for the frozen *Artemia* diet. Thus, as was observed for larval American lobsters (Fiore and Tlusty, 2005), CAR2 is suitable as a partial substitute for frozen *Artemia*. Again, because of CAR2's low cost, feeding it with or without frozen *Artemia* is more cost-effective than frozen *Artemia* alone.

Growth of early benthic juvenile lobsters in the first two experiments described here was less than that previously reported during research into formulated diet development for American lobsters (Conklin et al., 1980; D'Abramo and Conklin, 1985; Bordner et al., 1986). The lower growth observed here can be partially attributed to hatchery conditions; system water was not heated to a temperature conducive to maximal growth rates (Koshio et al., 1989). However, a potentially more significant factor in the reduced growth rates observed in this experiment was the juvenile lobsters' limited access to food. While other studies provided lobsters with near-constant access to food, here feeding time was limited to 4 to 6 h per day. Bordner and Conklin (1981) found that starved juvenile lobsters only consumed 34% of their daily intake in the first 4 h of feeding, and that lobsters that consumed less food per week had growth that was reduced in proportion to total food consumption. The mean 3-month weights of the frozen *Artemia*-fed lobsters in experiments One and Two (293 mg for "B" and 303.5 mg for "0/7") were approximately half of the mean 100-day weights of frozen *Artemia*-fed intact juvenile lobsters (571 mg at 15 °C and 648 mg at 20 °C) (Koshio et al., 1989). This growth difference would be consistent with lobsters in experiments One and Two consuming roughly half the amount of diet as those lobsters which had 24-h per day access to feed. Therefore, if feeding time for the diets studied

here were increased, growth would be expected to increase proportionally.

The inferior performance of the CAR1 and CAR3 diets relative to the *Artemia* control indicates that they may be lacking in some essential nutrient(s). The prevalence of molt related difficulties observed in lobsters fed CAR1 and frozen *Artemia* diets is reflective of a dietary nutrient deficiency, yet the nature of the deficient nutrient(s) is likely to differ between these two diets. CAR1-fed lobsters showed low growth as well as increased molt related difficulties. In contrast, while *Artemia*-fed lobsters also had molt related difficulties, they had relatively higher growth suggesting the deficient nutrient(s) were expressed at the higher growth. In the Second experiment, the frozen adult *Artemia* appeared to be of low quality, and the nutrient deficiency was expressed as a decrease in survival. CAR3 appeared to be a nutritionally complete diet as evidenced by the high survival and low incidence of molt death syndrome (MDS) of juveniles in that group. Poor growth in this group may have been a result of small particle size leading to decreased total ingestion by lobsters, even though wild early benthic juvenile lobsters are believed to utilize plankton as a significant portion of their diet (Lavalli, 1991). Future testing of artificial diets will need to focus on larger particle sizes, and retesting a larger particle size of the CAR3 diet for its suitability in ongrowing juvenile lobsters would be worthwhile. Overall, these results indicate that while artificially formulated diets developed for other species can be used to grow lobsters, care needs to be exercised in the selection of diets. Even diets specifically formulated for other crustaceans can be unsuitable for rearing American lobsters.

CAR2 provided a suitable feed for lobsters up to two years of age, although this diet had to be bound by gelatin to make it more appropriate for consumption by larger animals (>15 mm CL). Lobsters fed this diet in experiment Three exhibited higher survival, equivalent weight gain and blood chemistry, greater condition factor, thicker carapaces, and less external fouling than animals fed frozen adult *Artemia*. Animals fed G diet exhibited a poorer performance than those fed B and E diets. The G diet contained 35% crude protein, compared to 50 and 57% for the B and E diets, respectively. Reported protein requirements of lobsters vary from a low of 20% (Capuzzo and Lan-

caster, 1979) to a high of 60% (Castell and Budson, 1974), yet information regarding the nutritional requirements of lobsters between 1 year of age and maturity (approximately 7 years) are still quite nebulous. The high protein and low cost of CAR2 make this a preferable diet over frozen *Artemia* as a diet for one to two year old American lobsters. The poor performance of animals fed the low protein G diet potentially suggests that one year old lobsters may still require high protein diets. Nonetheless, other causative reasons for the difference in performance (e.g. contamination, degradation or spoilage of one of the diet components) cannot be ruled out. Hemocytic enteritis in some decapod crustaceans, such as was detected in 2 of the 3 G animals examined at 150 days, may be caused by *Vibrio* infection, or perhaps by a lipopolysaccharide endotoxin released in the gut during digestion of filamentous cyanobacteria (Lightner, 1988). Survival in the G group was much lower than that usually achieved in this facility on the same formulation (Tlusty, unpublished data).

CAR diets offer benefits over live and frozen *Artemia* beyond just their reduced cost. Since they are processed, these feeds would tend to be of more uniform quality between lots compared to products consisting of live food that has been frozen. The variation in survival between the *Artemia*-fed lobsters in experiments One and Two is a good example of what can be expected when frozen *Artemia* is used as a diet, as the variable quality occurred between lots purchased from the same supplier. From a practical standpoint, CAR diets would also be easier to store and manage than natural foodstuffs (Lim et al., 1997). A second benefit is that juvenile lobsters fed formulated diets low in carotenoid pigments will be a pale to translucent white color (D'Abramo et al., 1983; Tlusty and Hyland, 2005). Previously, blue lobsters were examined for their potential as an enhancement tool and as a viable means for identifying hatchery-raised animals in the field (Beal et al., 1998). While hatchery-raised blue lobsters appeared to behave normally upon release into the wild, laboratory experiments indicated that they were more susceptible to predation particularly on sandy substrates (Beal et al., 1998). Colorless lobsters may be a more beneficial color morph to release. During and shortly after release, juvenile lobsters are vulnerable to predation. If colorless lobsters are less visible to predators, then more

may be able to survive until they find suitable shelter compared to colored lobsters. As pigments accumulate from the post-release natural diet, these juveniles would subsequently acquire coloration appropriate to their new environment. It remains to be tested if this colorless phase of American lobsters could be successfully integrated into an enhancement program. A third benefit is that formulated diets may produce an overall healthier animal. Stewart et al. (1967) observed that wild lobsters had higher serum protein values than captive-fed or captive-starved animals. Older juveniles fed the B and E diets had higher refractive indices than did animals fed the G diet potentially indicating higher nutritional quality of the E and B diets. This result was further supported by the lipid quantity in the mid-gut gland. While increased hemolymph glucose is a response to stress in lobsters (Prince et al., 2002), glucose is also a precursor to chitin. E-fed lobsters which had the highest glucose concentration also tended to have the thickest cuticles. Further experimentation is required to clarify this relationship between baseline glucose level and lobster health. Lobsters fed the CAR2-gel diet (E) had less external fouling than did those lobsters fed the other two diets. The culture of live animals for feeding larval and juvenile lobsters was proposed as a likely route for increased incidence of bacterial contamination (Tlusty et al., 2001; Cox and Johnston, 2003) since *Artemia* is a known vector of juvenile lobster pathogens, including *Fusarium*, *Haliphthoros*, and *Vibrio* (Fisher et al., 1978; Rosemark and Conklin, 1983; Fisher, 1988). Pathogen exposure in combination with deficient nutrition as was observed with the 100% frozen *Artemia* diet in Experiment 2 could prove to be a lethal combination for juvenile lobsters in a hatchery setting.

5. Conclusion

While lobsters fed 100% CAR diets did not grow as rapidly as those fed frozen *Artemia*, lobsters fed CAR diets did outperform *Artemia*-fed lobsters on other metrics, namely condition factor, and overall economic benefit. When lobsters are being reared for enhancement, as is the case for most lobster hatchery programs today (Beal et al., 1998), simple goals such as maximum size or economic minimization may not be de-

sirable. In enhancement programs, the desired output is an animal that will grow and survive when released to the wild. If increased condition factor is used as a metric of potential success when an animal is released in an enhancement program (Farmer, 1994), then the CAR2 based diets tested here would be a preferred choice for an enhancement program. It is clear that feeding a diet solely consisting of frozen *Artemia* to early juvenile lobsters may result in variable performance depending on food quality which may ultimately lead to poor survival in the wild. The performance of CAR diets in these experiments warrants further investigation of the suitability of these diets to rear animals that have a high probability of realizing desirable growth and survival when released into the wild.

This study demonstrated that CAR diets were suitable in the hatchery rearing of juvenile American lobsters, from stage IV up to two years of age. While the growth rates achieved would be inadequate if the goal were to produce market-sized lobsters, this work demonstrated growth on the CAR2 diet similar enough to a frozen *Artemia* diet to warrant further evaluation of its utility for aquaculture production of juvenile lobsters for enhancement programs. Increasing feeding time and culture temperatures would be expected to increase growth rates to a level that is acceptable in production of releasable juveniles. The level of survival observed in this study was equivalent to that experienced by programs currently enhancing the European lobster (values in Nicosia and Lavalli, 1999). As food is the second most expensive production factor, accounting for approximately 30% of the total cost (Conklin and Chang, 1993), a decrease in food costs as reflected in this study would result in significantly lower costs of production. Making hatchery production more cost-effective could lead policy makers to be more prone to reconsider the integration of American lobster enhancement as a potential management tool. The success of feeding juvenile lobsters off-the-shelf CAR diets is part of an initial step in determining the feasibility of enhancement programs for American lobsters. Still, further work will need to investigate the timing and strategy of release of juvenile animals, as well as documenting their survivorship in the wild. Based on the success of European enhancement programs, the augmenta-

tion of wild populations of American lobsters is feasible and quickly becoming more cost effective.

Acknowledgements

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