Hatchery performance of early benthic juvenile American lobsters (*Homarus americanus*) fed enriched frozen adult *Artemia* diets

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Abstract

One of the main factors hindering aquaculture production of American lobsters (*Homarus americanus*) is the need for a cost-effective and nutritionally sound diet. Live *Artemia* results in good growth, but is expensive and is a constant source of contamination. Frozen *Artemia*, although lower in cost, generally results in decreased survivorship and growth relative to live *Artemia*. The recent advent and mass production of enriched frozen *Artemia* products may provide for a cost-effective and nutritionally complete food source for culturing American lobsters. Here, commercially available frozen adult *Artemia* enriched with either n-3 fatty acids, or *Spirulina* was fed to juvenile American lobsters, and their growth and survivorship for three months was compared with that of animals fed unenriched frozen adult *Artemia*. Both enriched *Artemia* products produced survivorship superior to that for animals fed unenriched *Artemia*. Results for growth were equivocal although animals fed the *Spirulina*-enriched *Artemia* had the greatest condition factor. Although more costly by the end of the experiment, enriched diets were more economically efficient than unenriched *Artemia*. This research demonstrates that enriched feedstuffs are cost-effective over longer time intervals, and benefits may continue beyond the hatchery-rearing phase. Enriched diets may also yield animals with a better condition factor, which may further influence their survivorship when released to the wild in enhancement programmes.

KEY WORDS: American lobster, condition factor, frozen *Artemia*, n-3, *Spirulina*, stock enhancement

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Introduction

For many commercially important species, the interest in aquaculture production for stock enhancement or food is often inversely proportional to fishery landings. American lobsters (*Homarus americanus*, H. Milne Edwards) were first cultured in captivity in the late 1800s, when wild stocks experienced their first decline (Aiken & Waddy 1995; Nicosia & Lavalli 1999). In the subsequent century, aquaculture of American lobsters was often attempted, but was never sufficiently economically profitable to make commercial production feasible (Bannister & Addison 1998; Nicosia & Lavalli 1999). Current efforts remain marginal at best, with only a few seasonal or small-scale research hatcheries in existence (Beal *et al*. 1998; Goldstein & Tlusty 2003). However, a recent decline in landings at the southern extent of the species' range (Richardson 2003), coupled with the general concern that the stock is overfished throughout its range (Atlantic States Marine Fisheries Commission 2000), could result in renewed interest in aquaculture production of American lobster.

Although the North American effort to farm Homarid lobsters has not proven economically feasible, European lobster enhancement programmes have fared substantially better (Bannister & Addison 1998). Norway represents the best case study where in 1997, 43% of *H. gammarus* landings and 73% of pre-recruits were of hatchery origin (Agnalt *et al*. 1999). The difference between the North American and European programmes was in the age and number of animals released (Bannister & Addison 1998). In North America, the strategy has been to release large numbers (*c*. 500,000 annually per hatchery) of early benthic stage lobsters (Nicosia & Lavalli 1999). European hatcheries by contrast, have typically released fewer (*c*. 100,000 annually per hatchery), much later stage juveniles (Bannister & Addison 1998). If production of American lobsters is to increase, the

overall strategy for American lobster stock enhancement programmes needs to be refocused based on the success of European efforts. In particular, methods to cost-effectively rear older American lobster need to be developed.

Many of the problems that have hindered American lobster aquaculture initiatives of the 1970s/80s have been resolved in other sectors of the rapidly growing global aquaculture industry. These problems have included insufficient technology for hatchery equipment, broodstock management techniques, and rearing methodology (Conklin & Chang 1993; Aiken & Waddy 1995). The one deficit that has yet to be resolved is the creation of commercial diets specific to American lobsters that are nutritionally complete and economically feasible to produce (Aiken & Waddy 1995). The few studies that have evaluated such diets have focused on those developed for market sized, adult animals and yielded equivocal results (Bayer & D'Agostino 1980; Gallagher *et al*. 1982; Bordner *et al*. 1986).

Traditionally, live adult *Artemia* has been used as the staple diet for culturing larval and juvenile lobsters (Conklin 1995). Although this diet yields the highest survival and growth rates in hatchery settings (Conklin 1995), producing an adequate supply for large-scale hatchery applications has proven expensive and effort intensive (Leger *et al*. 1986). Live foods also constitute a constant source of bacterial contamination and potential disease vectors (De Wolf *et al*. 1998; Cox & Johnston 2003). As an alternate to live *Artemia*, a combination of frozen *Artemia* and unfiltered seawater containing local plankton can result in reasonable growth and survivorship (Lavalli 1991). Unfortunately, increased incidence of disease and pollution in coastal waters makes the use of unfiltered seawater risky. It therefore remains desirable to find a diet that provides complete nutrition in a closed, filtered seawater system.

Although frozen *Artemia* is commercially available at a fraction of the cost of live *Artemia*, growth and survivorship of lobsters fed frozen adult *Artemia* is approximately 60% of that in lobsters fed live *Artemia* (Conklin 1995). Recently, frozen *Artemia* that have been fed an enriched diet just prior to harvest and freezing have become commercially available. These enriched forms have an increased nutritional value compared with the unenriched product. The enrichment process creates a food that is intermediate in cost between unenriched frozen, and live *Artemia*, although its benefits as a diet for hatchery reared American lobsters remains untested. Thus, the aims of this investigation were to (i) determine if the increased benefit of enriched frozen *Artemia* diets is proportional to their increased cost when compared with unenriched frozen adult *Artemia*, and (ii) if these relative benefits change with the two opposing enhancement rearing strategies (many small vs. fewer larger animals) which have traditionally been used in the stock enhancement and culturing of Homarid lobsters.

Materials and methods

Facility and animal source

Experiments were conducted in a small-scale American lobster research hatchery at the New England Aquarium (Boston, MA, USA). A single gravid female American lobster was housed in a $62 \times 38 \times 22$ cm incubation tub within a semi-closed flow through seawater system (15–18 °C; mean salinity = 32.0 g L^{-1}; pH = 7.84–7.97; $NH_4 < 70$ mg L^{-1}). At hatching, first-stage larvae were collected from a catch screen and transferred to two separate planktonic kreisels (40 L cylindrical tanks with turbulent, upwelling water flow, based on Hughes *et al*. 1974) within a semi-closed flow through filtered sea water system (same parameters as above). All animals were subjected to a daily artificial light cycle (13–11 LD). Stages I and II larvae were fed live 48-h-old *Artemia* nauplii enriched with Super Selco® (INVE Aquaculture nv, Dendermonde, Belgium). The diet of stage III larvae also included live adult *Artemia* and frozen n-3 enriched *Artemia*. Larvae and postlarvae in both kreisels were fed *ad libitum* three to four times daily.

Experimental design and setup

Experimental animals were randomly placed within one of three $33 \times 22 \times 5.5$ cm black plastic 'condo trays'. Each condo tray contained 24 separate compartments where the individual lobsters resided. All condo trays were permeated with holes and lined with mesh-screening to allow ample flushing and circulation of seawater throughout each compartment. Substrate, consisting of a few pieces of gravel and an identifying plastic letter bead was placed in each compartment. Stage IV post-larvae were collected from the kreisels over three consecutive days until there were a total of 24 lobsters in each test group. Siblings were used to minimize variation in embryonic development conditions and to avoid genetic variability in growth patterns (Hedgecock & Nelson 1978). Post-larvae from each day and each kreisel were evenly distributed among the three test groups. Missing appendages were noted, and those post-larvae missing one or more eyestalks were not used; eyestalk ablation induces premature moulting, abnormal body proportions and pale coloration (Koshio *et al*. 1992). Each post-larva was first

blotted dry and weighed on an OHAUS Galaxy 160 scale (OHAUS Corp., Pine Brook, NJ, USA), and then digitally photographed with an Intel QX3 digital microscope (Intel Corp., Santa Clara, CA, USA). Photographs were exported to a personal computer as JPEG images for later determination of carapace lengths. Post-larvae were placed individually into the condo tray compartments, and the condo trays were placed side by side in an 80 L, $193 \times 53 \times 7.5$ cm fibreglass seatray on the same recirculating seawater system as the kreisels. Condo tray positions in the seatray were rotated daily to minimize the impact of any possible positional effects on water flow.

Test diets

Each condo tray was assigned one of the three test diets and juvenile lobsters were fed only their designated test diet for the duration of the experiment. Three frozen adult *Artemia* diets were purchased directly from San Francisco Bay Brand (San Francisco, CA, USA), and included unenriched (UN), n-3 enriched (O3), and *Spirulina*-enriched (SP) frozen adult *Artemia*. Each diet was prepared similarly. An 8.3 g sample of the test *Artemia* (stored at −70 °C) was placed in a beaker with approximately 100 mL of filtered seawater at 20 °C to thaw. The thawed brine suspension was then fed to post-larvae by pipetting two drops (approximately 0.045 g brine, wet weight) into each lobster's compartment. Leftover thawed brine was refrigerated and used on subsequent days. Test diets were administered over a 90-day experimental period.

Measurements and analyses

All test animals were censused daily for moulting, mortalities, missing appendages, and any other miscellaneous observations (i.e. abnormal posture or activity level). Growth (weight and carapace length) was measured on days 28 and 90, germane to the two strategies of potential lobster enhancement (many small animals or fewer older animals). Carapace length was determined from digital images using an automated MATLAB® program. A condition factor was calculated for each lobster based on a modified version of the von Bertalanffy growth equation (q = wt CL^{-3}) (Sparre & Venema 1998). The condition factor 'q' for any individual animal is proportional to its weight (g) divided by the cube of its carapace length (CL). Calculated biomass per treatment was a sum of each individual's weight. To assess the relative value of each of the various diets used and their resulting growth and survivorship benefit for early juvenile lobsters, benefits and costs were graphically analysed and modelled. The benefit of each diet relative to the performance of animals fed the unenriched frozen *Artemia* diet (UN) was plotted as a function of day. UN was designated as the reference diet as it has been previously used for the hatchery production of lobsters (Conklin 1995), while the O3 and SP diets were the new diet treatments being tested. Costs were set relative to the UN diet and plotted as reference lines graphically. Enriched diets were considered to be economically beneficial compared to the UN reference diet when the relative benefit curve exceeded the relative cost reference line ($B/C_{\text{test diet}} > B/C_{UN}$). Statistical analyses on individual data (growth, carapace length and condition factor) were conducted on the 28 and 90-day samples, again applicable to the two possible scenarios of either stocking many smaller animals or fewer older animals. Intermoult intervals for moults to stages V and VI were also analysed as a function of diet treatment. This experiment was designed as a two-factor repeated measures ANOVA with the factors being diet (three levels) and measurement period (two levels); however, low survivorship incurred by lobsters fed the UN diet precluded complete analysis. Thus, data were analysed as one-way (diet) ANOVAs with separate analyses being conducted on each sample period, with *post hoc* pairwise comparisons made using a series of Tukey's HSD tests. Untransformed data were used provided it met assumptions of normality, independence and equal variance. Data that were not normal or had unequal variances were analysed as a Kruskal–Wallis ANOVA on ranked data. In all cases of non-normal data, the analysis of ranked data did not change the interpretation; hence all reported values reflect the mean + 95% CI (confidence interval) of untransformed data. For results with P-value $0.05 > P > 0.01$, power was calculated.

Results

There were no initial treatment differences the in lobsters' carapace length (one-way ANOVA, $F_{2,67} = 0.561$, $P > 0.50$), weight or condition factor (Kruskal–Wallis one-way ANOVA, d.f. = 2, $H = 0.52$, $P > 0.70$, $H = 1.56$, $P > 0.40$ respectively). Enriched *Artemia* diets had a large influence on the survivorship, but a diminished impact on the growth of juvenile American lobsters. Animals fed SP had the highest survivorship with 87.5% surviving to 90 days (Fig. 1). Animals fed O3 were intermediate with 46.8% survivorship, while only 8.3% of those fed UN survived 90 d. Similarly, the mortalities in this experiment exhibited diet treatment differences in the percentage of deaths that occurred during moulting. The UN treatment had the greatest rate of death

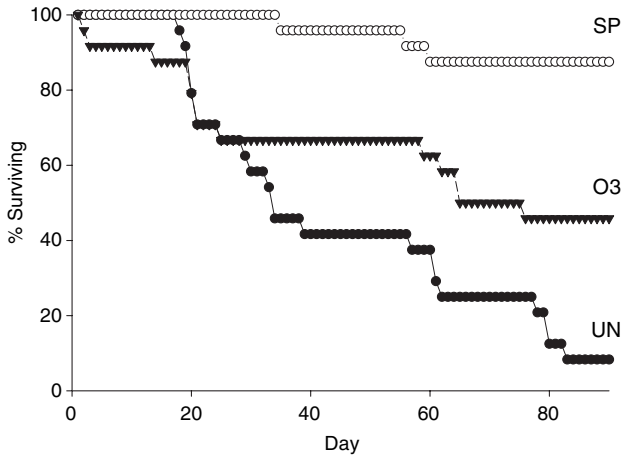

Figure 1 The percent of animals surviving per day when fed one of three experimental diets. The diets were unenriched frozen adult *Artemia* (UN), n-3 enriched frozen adult *Artemia* (O3), or *Spirulina*-enriched frozen adult *Artemia* (SP). The experiment began with stage IV post-larvae, and 24 animals were used per diet treatment.

during moult (59.1%), followed by O3 (38.5%) and SP (33.3%). While there were large differences in survivorship, growth provided equivocal results. There were no diet treatment differences in the percent increase in body weight over 28 (Kruskal–Wallis one-way ANOVA, d.f. = 2, $H = 4.251$, $P > 0.10$), or 90 days (one-way ANOVA, $F_{2,31} = 3.279$, $P > 0.05$; Table 1). The increase in carapace length of animals in the O3 treatment was greater after 28 days than those in the SP treatment (one-way ANOVA, $F_{2,53} = 3.757$, $P < 0.03$, power = 0.513; Tukey's HSD test, $q = 3.725$, $P < 0.05$, Table 1), while the UN treatment was statistically similar to both the O3 and SP results (Tukey's HSD test, $q < 3.296$, $P > 0.06$, Table 1). By 90 days, treatment differences in % increase in carapace length were not apparent (one-way ANOVA, $F_{2,31} = 0.181$, $P > 0.80$).

Diet did not significantly influence the number of days it took animals to reach the moults to stages V and VI (Kruskal–Wallis one-way ANOVA, d.f. = 2, fifth, $H = 0.86$, $P > 0.6$; sixth, $H = 2.90$, $P > 0.2$). Although there were only minor treatment differences in growth and moulting, diet had a significant impact on the condition factor of animals (one way ANOVA; 28 days, $F_{2,53} = 8.96$, $P < 0.001$; 90 days, $F_{2,31} = 7.34$, $P < 0.002$, Table 1). For both time periods, the condition factor of animals fed SP was significantly greater than animals fed O3 (Tukey's HSD test, 28 days, $q = 5.99$, $P < 0.001$; 90 days, $q = 4.86$, $P < 0.005$, Table 1), but there were no statistical differences between SP and UN, or O3 and UN (for all cases Tukey's HSD test, $q < 3.38$, $P > 0.05$). These trends in statistical significance suggest that large increases in biomass for the SP and O3 treatments coupled

Table 1 Growth parameters of juvenile American lobsters fed one of three frozen adult *Artemia* diets

	28 Days			90 Days		
	SP ($n = 24$)	O3 ($n = 16$)	UN ($n = 16$)	SP ($n = 20$)	O3 ($n = 11$)	UN ($n = 3$)
% increase weight day^{-1}	$3.24^{a} \pm 0.59$	$2.45^{a} \pm 0.28$	$2.60^{a} \pm 0.35$	$4.61^{a} \pm 0.54$	$3.53^{a} \pm 0.56$	$3.39^{a} \pm 2.54$
% increase CL day^{-1}	$0.51^{a} \pm 0.16$	$0.82^{b} \pm 0.16$	$0.55^{a,b} \pm 0.15$	$0.68^{a} \pm 0.08$	$0.73^{a} \pm 0.16$	$0.65^{a} \pm 0.33$
Condition factor	$0.00051^{a} \pm 0.000042$	$0.00039^{b} \pm 0.000030$	$0.00046^{a,b} \pm 0.000037$	$0.00049^{a} \pm 0.000043$	$0.00038^{b} \pm 0.000037$	$0.00037^{a,b} \pm 0.000044$

Animals were measured at both 28 and 90 days after they were placed on experimental diets (unenriched frozen adult *Artemia* (UN), n-3 enriched frozen adult *Artemia* (O3), or *Spirulina*-enriched frozen adult *Artemia* (SP). Statistical tests were conducted separately on each growth parameter for each measurement period. Statistical similarity was measured by a one-way ANOVA or Kruskal–Wallis ANOVA on ranked data was denoted by identical superscripts. Nonparametric analyses of the data did not influence the interpretation of the results, hence mean ± 95% CI were presented here.

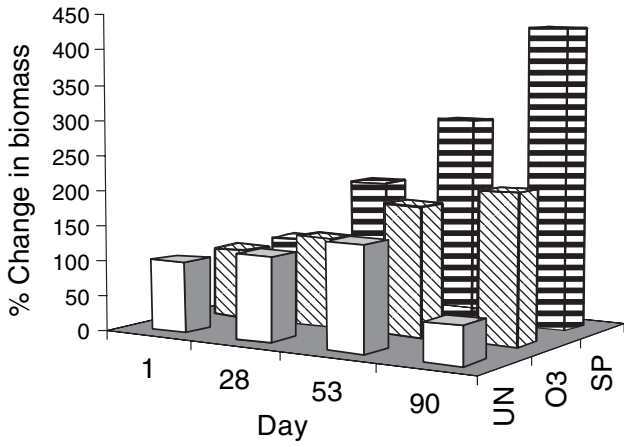

Figure 2 The % change in biomass within each treatment for juvenile lobsters fed one of three frozen adult *Artemia* diets.

with a loss of biomass for the UN treatment (Fig. 2) were largely determined by differences in the number of survivors, not because of differences in growth rates.

While the benefit (biomass and survivorship) of SP was greater than O3 or UN, associated costs exhibited an opposite trend. The UN diet was the cheapest at \$1.97 kg^{-1} (wet weight basis), O3 was intermediate (\$2.98 kg^{-1}), and SP was most expensive (\$7.87 kg^{-1}). Animals fed O3 would have to perform 151% better than those fed UN for this diet to be more cost-effective. Lobsters fed SP would have to perform even better, with the break-even point for this diet at a 399% increase in performance. Comparing the relative survivorship of lobsters by diet type, the O3 diet outperformed the unenriched diet after day 39, while the SP treatment did not out perform UN until day 78. The relative biomass curve of the O3 treatment was shallower than the relative number curve, and thus it took more days (64) for this diet to exceed the cost curve. The relative biomass curve for the SP diet was steeper than the relative number curve, and therefore took less time for the biomass of the SP treatment (70 days) to exceed the performance of the UN treatment.

Discussion

The better growth and survival of juvenile lobsters on *Spirulina*-enriched food confirms and corroborates results observed for a diverse array of marine organisms including abalone *Haliotis asinine* (Bautista-Teruel *et al*. 2003), Korean rockfish *Sebastes schlegeli* Hilgendorf (Cho *et al*. 2001), seahorses *Hippocampus abdominalis* (Woods 2003), shrimp, *Farfantepenaeus paulensis* (Thompson *et al*. 2002), and yellowtail tuna *Tunnus albacares* (Cyanotech 2003) as well as some fresh water species, notably tilapia *Oreochromis niloticus* (Lu *et al*. 2002) and common carp *Cyprinus carpio* L. (Nandeesha *et al*. 1998). In the case of lobsters, *Spirulina*-enriched frozen *Artemia* appeared to most significantly impact survivorship. While diet affected few growth parameters, the condition factor of animals fed a *Spirulina*-enriched diet was greater than that of animals fed n-3 enriched diet. There is the possibility that growth was imperceptibly lower in the *Spirulina* treatment leading to increased condition factors. Although the mean carapace length of lobsters fed the n-3 diet was significantly longer at 28 days than in lobsters fed the other two diets, this may be the result of differences in how many lobsters in each group moulted before or after they were measured. By 90 days, this result was no longer evident. While the additional n-3 oil may have assisted with the animals' moulting capabilities (e.g. fewer deaths during moulting compared with the unenriched diet), it did not impact condition factor.

Poor performance of lobsters fed the unenriched diet was exemplified by their low survivorship to 90 days. One caveat is that it is not known how repeatable this result would be with different lots of *Artemia*. Individual lots of frozen *Artemia* can be highly variable reflective of their ever changing environmental culturing conditions such as dissolved organic materials, water quality, nutritional condition and bacterial flora (Leger *et al*. 1986). Standard microbial analysis indicates that samples of frozen brine shrimp can vary significantly with respect to fluctuating and diverse numbers of marine bacteria, yeasts and even coliforms from where they were harvested (Leger *et al*. 1986). Experiments recently conducted at the New England Aquarium observed a 67 and 28% survivorship over a 90-day period for animals fed different lots of n-3 enriched *Artemia* (M.M. Tlusty, D.R. Fiore and J.S. Goldstein unpublished data). One potential benefit of using brine enhancements is that they can assist in decreasing the variability between lots, although these three survivorship values of n-3 enriched *Artemia* were still lower than that of the animals fed *Spirulina*-enriched *Artemia*, suggesting *Spirulina*'s overall utility as an enhancement product.

This experiment did not include a causal investigation of the mechanism that resulted in better performance by animals fed *Spirulina*-enriched adult *Artemia*. *Spirulina* may provide numerous benefits as an enriching agent. For example, proximate analyses indicate that *Spirulina* is high in n-6 fatty acids, but also includes a number of n-3 species, vitamins, minerals, and enzymes (Aquafauna 2004). The n-3 enriched *Artemia* are fed menhaden oil which is high in DHA and EPA. High levels of phospholipids and carotenoid

pigments that are found in *Spirulina* (*Artemia* International 2003) can have significant effects on overall lobster health. Phospholipids are required in lobster diets, especially to prevent a condition in early juveniles known as moult-death syndrome (Kean *et al*. 1985; Bordner *et al*. 1986), one of the leading complications for inadequately fed hatchery raised lobsters. While insufficient carotenoids lead to an abnormal pale blue shell coloration, a paucity of this nutrient may also lead to inferior health (Bordner *et al*. 1986; Lim *et al*. 1997). In other animals, *Spirulina* may enhance immune system functioning (Belay *et al*. 1996; Duncan & Kiesius 1996), which subsequently reduces the need for medicated treatments (Henson 1990; *Artemia* International 2003). *Spirulina* also heightens digestive enzymes (Cyanotech 2003; *Artemia* International 2003) and contains high levels of antioxidants (Far East Bio-Tec 2003), which may prevent degradation and loss of essential fatty acids such as phospholipids in food during storage.

The intricacies of this economic model raise an important distinction for the production of juvenile lobsters – defining the goal of the programme. Often, enhancement decisions are purely economic, with the goal being to maximize production while minimizing costs. While it is important to determine economic break even points for each diet, simple economics will not determine the success of an enhancement programme. If a lobster enhancement programme called for release of animals after short term (e.g. 14 days) rearing, then under the production scenarios presented herein, unenriched frozen *Artemia* would appear to be the diet of choice. Yet, animals fed this diet had lower long-term survivorship than those fed other diets, and additional research suggests that the diet of a lobster early in the production cycle influences its subsequent survivorship (Fiore & Tlusty 2005). Similarly, initial analysis (using price determined at point of purchase) suggested n-3 enriched *Artemia* was the most economical means to grow juvenile lobsters. Yet, the condition factor of lobsters fed n-3 was no better than that of lobsters fed unenriched *Artemia*. It is unknown whether condition factor is a critical component for success in a lobster enhancement programme, while it does appear to be a factor in Atlantic salmon enhancement (Farmer 1994). Identification of measurable parameters predictive of post-release success would be of great utility in improving hatchery methods and returns on enhancement efforts. Therefore, further development of American lobster stock enhancement programmes should consider how condition factors influence survivorship of juvenile lobsters released to the wild. In the time since this experiment was conducted, the cost of *Spirulina*-enriched frozen adult *Artemia* has decreased significantly, to the point that it is now equivalent in cost to n-3 enriched. Using a new relative cost of 151% (the dotted horizontal reference line in Fig. 3), then lobsters fed the *Spirulina* diet would exceed the 1 : 1 benefit: cost break even point much sooner than would lobsters fed the n-3 diet.

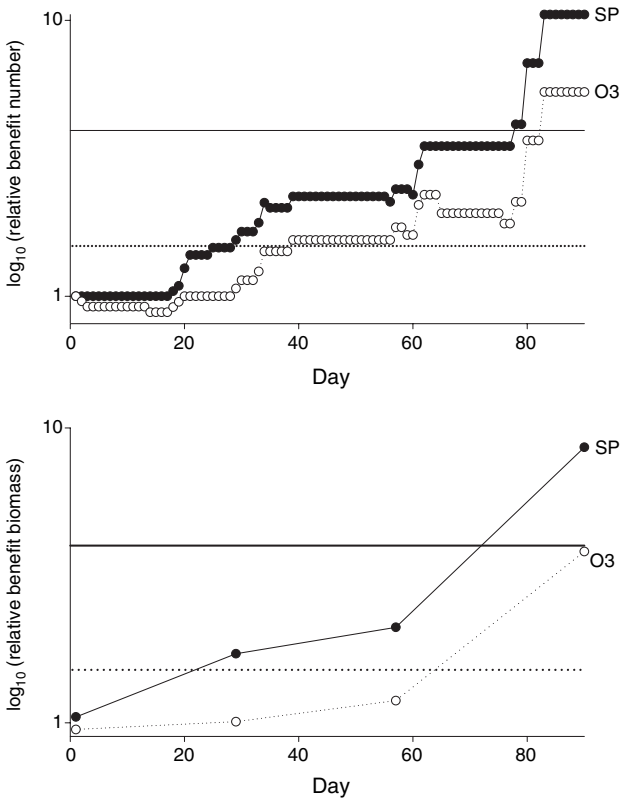

Figure 3 The cumulative gain function for the relative benefits in terms of numbers (top) or biomass (bottom) of juvenile lobsters fed n-3 (dotted line) or *Spirulina* (solid line) compared with the performance of animals fed an unenriched frozen adult *Artemia* diet. The horizontal reference lines designate the relative cost for each diet. The test diet is more economical than the reference diet when the relative benefit lines exceed the relative cost line.

Conclusion

In summary, the survivorship and growth of American lobsters is greatly influenced by diet. While short-term analyses may indicate enhanced feedstuffs are uneconomical, enriched diets may be cost-effective for enhancement programmes on longer temporal scales. They may also be more effective if the body condition created through the feeding of the diet aids the animal in surviving post-diet treatment, such as when they are reared to be released into the field. In this experiment, pronounced effects of enhanced diets were apparent after a minimum of only 14 days. Animals fed *Spirulina*-enriched

frozen adult brine shrimp had higher survivorship, condition factor, and total biomass than animals fed the other two diets. Further investigation is warranted to determine if significant differences in condition factor of hatchery-produced juvenile Homarid lobsters affects post-release success in enhancement programmes.

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