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1980 LOBSTER NUTRITION WORKSHOP PROCEEDINGS

Editors
Robert C. Bayer
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OPERATION OF A LOBSTER POUND IN MAINE

Herbert O. Hodgkins

In Maine, nearly 4 million lobsters are held in pounds annually beginning in the fall when lobsters are plentiful and the price is low. These are generally soft shell lobsters which are held and fed until winter or early spring and sold as hard shell lobsters, when prices are higher. At Tidal Falls, when the pound is stocked an attempt is made to limit the number of "cull" one-claw lobsters so that mainly high quality two-claw lobsters are sold.

A lobster pound is a small cove that has a dam in place that allows tidal exchange of water. Many pounds also run aeration systems to keep oxygen levels high. Lobsters are removed from the pound with a drag or with an air bubble hydraulic pump operated by a diver. Herring cuttings, the scrap from canneries, is the dominant feed used for lobsters held in pounds. It is fed at a rate of 60 lbs. (one bushel) per 5,000 pounds of lobsters daily. Less is fed as temperature drops affecting lobsters' feeding intake, with no feeding done when the pound freezes over or temperature drops to around 0°C. Feed consumption is monitored by a diver or by placing a quantity of feed at the edge of the pound where it can be observed.

The pound is served by 9 - 10 lobster boats. In return, the pounds has available a reliable supply of bait and gasoline, and fishing supplies can be bought from it. In other harbors, where there are a number of buyers, there is much competition for the lobsters. Tidal Falls lobster pound has no retail trade; it sells lobsters in large volumes to companies which grade the lobsters according to size and redistribute them by air or truck to restaurants and seafood retailers.

A COMMERCIAL VIEW OF LOBSTER NUTRITION

Philip L. Wilson III

Aquaculture Enterprises began looking at the feasibility of lobster culture in 1972. Synthetic diets were tried for three years and abandoned. The approach was changed to trying any food or feed by-product that could be obtained for under 20¢ per pound. Feeds such as discarded cattle from feed lots, corn, liver, fish racks, etc., were used. Underutilized species such as mussels or sea urchins were not used since they are not available in commercial quantity. Only waste products from million pound fisheries are used to assure supply. A homogenized diet with some variation in constituents seems to work best. Lobsters appear to have best appetite when fed a diet that is varied in its makeup. In a large scale facility ingredients would be stockpiled in a freezer.

Last year lobsters were raised from egg to market size using only natural ingredients as described. Growth rates compare favorably to those obtained on brine shrimp. To be competitive in the California sales market, production costs must be kept below \$5.00/lb. Due to the cost of labor, overhead, and freezing, and to lobster conversion rates, the food cost must be kept below 25¢/lb.

For rapid growth and lower cost, elevated water temperatures are required. Aquaculture Enterprises was prepared to use either thermal effluent or tropical waters. They chose the former. At present they operate a pilot plant in Southern California, utilizing the effluent water from a Southern California Edison steam generating station. Data will be presented to Southern California Edison with a proposal to develop a million pound per year production facility on an adjacent site.

VITAMIN LEACHING IN LOBSTER RATIONS

Rex C. Infanger, Roger Mickelsen
Richard Heckmann, Sterling R. Wadley

INTRODUCTION

Commercial lobster production is not yet a reality. One of the many obstacles to lobster rearing is the development of suitable diets that promote adequate growth and produce low mortality rates. More than a dozen experimental diets have been formulated and tested at this laboratory. None of these diets, however, have produced growth rates greater than 90% of brine shrimp controls, and only one resulted in mortality less than the control. A problem encountered in feeding a formulated diet is that lobsters are nibblers and do not immediately consume all their food. The diets, after being placed in sea water, are degraded and nutrients leach from the feed.

Nutrient leaching of lobster rations is documented (Goldblatt *et al.*, 1978), and is a serious problem since the diet formulated is not the diet consumed. A series of experiments was designed to test water soluble vitamin leaching. Vitamins were added to diets in the form of an oil emulsion or by binding them in a protein matrix in an attempt to retard leaching. Five diets were formulated and tested in our laboratory. Diet 79-1 was used as a general baseline formula, diets 79-2-N and 79-2-E were modifications of 79-1 with margarine replacing the fish and vegetable oils. Diets 79-3 and 79-4 each had an added protein supplement with 79-3 also having hard fat and an emulsifier added. The diets were analyzed for moisture, crude protein, crude fat, ash and fiber. Vitamin analysis included leaching of thiamine, riboflavin, pyridoxine, and niacin. Two control groups designated as BS-1 and BS-2, were fed live brine shrimp, Artemia salina.

MATERIALS AND METHODS

Five diets were mixed and extruded for this experiment. They were hand mixed, with the lipid or protein fractions being hand rubbed in the process which causes a change in color and texture. When the diet mixes were consistent in color and texture, it was determined that the lipids or proteins were uniformly distributed. The diets were extruded through a Winger extruder at 12 lbs. pressure, with a die diameter of 2.5mm producing a pellet 3mm in diameter. Retention time in the machine was approximately 1.5 minutes with an internal temperature of about 60C. The extruded diets were cut in 20 cm lengths, sealed in ziplock bags and frozen. They remained frozen until they were fed to the animals.

The cost of these experimental diets is greatly influenced by expensive additions. Diet 79-1, the laboratory maintenance ration, costs approximately 42 cents per pound. Without the vitamin and mineral premix or the lecithin and

cholesterol it would cost about 11 cents per pound. This diet, when not being used in an experiment, is supplemented with live brine shrimp and/or squid, beef liver or beef heart and is fed to non-experimental animals.

The five diets were similar in composition, with the main difference being the type and amount of lipid added (Table 1). Diet 79-2-N differed from 79-2-E in that margarine and palm oil were added to the dry ingredients separate from the vitamin mix. Diet 79-2-E was made by melting margarine and palm oil together, the lecithin and cholesterol were then added to the oil followed by the vitamin mix. This was then mixed in a Waring blender and added to the dry ingredients.

Diet 79-3 was made by adding a hard fat mixture to the pre-mixed dry ingredients. The hard fat portion of this diet was formulated by melting solid cottonseed oil and emulsifier together. This mixture was agitated in a heated blender while a solution of warm water and vitamins was added. This combination was cooled and pulverized before adding it to the dry ingredients.

Diet 79-4 was produced by adding the vitamin mixture to whole, raw eggs. The eggs, which became a protein supplement, were cooled to set the proteins, ground in a Waring blender and added to the dry ingredients.

Each diet was fed once daily to a group of forty-four, L-6 to L-7 lobsters obtained from a single female. Excess food from the previous feeding was removed prior to the next feeding. Two control groups, one for each of the two identical water systems used in the experiment, were fed live brine shrimp daily. These water systems were designated as Tank 1 and Tank 2.

Samples of each diet were sent to a commercial laboratory for vitamin leaching analysis. Each diet was analyzed for thiamine, riboflavin, pyridoxine and niacin content initially and after two hours immersion in freshly made Instant Ocean. In a second analysis the water was analyzed for vitamins leached from the pellet after two hours. All tests were done in darkness to retard auto-oxidation.

Our laboratory tested salinity, nitrate, pH and temperature daily during the ninety day experiment in both water systems.

RESULTS

Data for animals fed the experimental diets are given in Table 2. Animals fed 79-1 had 41% mortality and increased 68% in length with an average of 0.051 mm growth per day. The surviving animals were 90% as large as the control group and had near normal coloration. It is possible that some live brine shrimp escaped past two screen barriers constructed between the control animals and those fed 79-1. This, however, was never observed but may account for the coloration.

Animals fed 79-2-N had poor coloration with a soft shell. They grew 0.053 mm daily, an increase of 69%, and had an 80% mortality. The lobsters on the

79-2-E diet had 52% mortality and grew an average of 0.046 mm per day, a 59% increase. The color of these animals was better than those fed 79-2-N.

The animals eating the hard fat diet, 79-3, increased in length 59% and had a mortality of 30%. This mortality figure is misleading because five of the animals included did not die but disappeared during a water fluctuation. These animals also had harder shell and better coloration compared with those fed 79-2 or 79-4. The animals fed 79-4 with protein supplementation developed harder carapace compared with all others fed synthetic diets. They increased 65% in length and had 16% mortality.

The two control groups had the best overall growth. The first, BS-1, had 98% carapace length increase, 0.074 mm per day with 25% mortality. Six deaths in this group occurred on one day when a return pipe became clogged dropping the water level below the cages. The second group, BS-2, had 20% mortality, a daily growth of 0.068 mm and increased 98% in carapace length.

The formulated diets had the following average analysis values: 25.9% moisture, 22.4% crude protein, 5.5% crude fat, 2.4% crude fiber, 5.2% ash, 38.4% carbohydrate, 2.9 Kcal per gram energy and a 0.08 protein energy ratio. The vitamin leaching data indicated an average of 82.8% thiamine, 30.2% riboflavin, 4.5% niacin and 32.3% pyridoxine leached from the diets in two hours.

Deviations of each diet from these mean values are summarized in Tables 3 and 4. Water quality in Tank 1 and Tank 2 was identical with the following results: pH = 8.0, salinity = 27.5 ppt, mean temperature = 21.7 C, and 0.0 ppm nitrite (Table 5).

DISCUSSION

Water soluble vitamins in all diets were reduced after two hours immersion in sea water. Thiamine leached out first with a 68% to 100% loss. Riboflavin and pyridoxine had two-hour leaching rates that projected a loss of 97% after 24 hours. Diet 79-4 was an exception with a projected 24 hour riboflavin loss of 93%. Niacin values remained comparatively high with a 50% loss in 24 hours.

Thiamine, in human nutrition, is important in carbohydrate metabolism, protein synthesis and nerve impulse transmission (Guthrie, 1975). This may also be true in lobster nutrition, particularly with carbohydrate metabolism. The inactivity of carboxylase and RNA transketolase in the experimental animals due to low thiamine levels may explain growth differences when compared with the controls. Less carbohydrate energy would be available for metabolism because of this deficiency.

The 79-4 diet produced the lowest mortality of any diet, including the controls. This may result from a higher level of phospholipids found in the protein supplement. Protein supplementation, however, did not reduce the vitamin leaching as expected. Initially, it was expected that cooking the egg would drive off the water trapping the vitamins in the secondary protein structure. A second protein supplemented diet was developed by aspirating the mixture of egg

and vitamins followed by a one hour cook in a steam retort. Table 6 indicates that this preparation method reduces the thiamine loss but increases the leaching of riboflavin, niacin and pyridoxine. The latter three compounds may not be complexing with the proteins in the diet.

Emulsification of vitamins in an oil and water matrix did retard vitamin leaching but did not stop it completely. It may be possible to slow leaching even more by dissolving the vitamins in water which is then incorporated in a water and oil emulsion. The original method simply whipped the vitamins in an existing water and oil emulsion.

The energy content of the diets appeared to have little influence on mortality rates. The protein energy ratios of all diets fell within recommended levels (Gallagher et al., 1979) indicating this was not the reason for reduced growth or high mortality. Diet 79-2-N and 79-3 had the same energy content, 3 Kcal per gram, but 79-2-N had the highest mortality and 79-3 had the second lowest mortality.

This study, when compared with other diet work, indicates that more research is needed on vitamin leaching. It appears, however, that a nutritionally adequate lobster ration needs a higher level of phospholipids and a lower protein energy level.

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Table 1 Diet composition.

Component	Diet Number				
	79-1 %*	79-2-N %*	79-2-E %*	79-3 %*	79-4 %*
Enriched Wheat Flour	45	47	47	46	48
Soybean Meal	11	11	11	7	7
Feather Meal	8	9	9	9	9
Poultry Meal	5	6	6	5	6
Menhaden Meal	5	6	6	5	6
Alfalfa Meal	5	6	6	5	6
Shrimp Meal	5	-	-	-	-
Protein Supplement	-	-	-	5	6
Torula Yeast	1	1	1	-	-
Brewers Yeast	1	1	1	2	2
Lecithin	1	1	1	1	1
Cholesterol	.5	.5	.5	.5	.5
Oleoresin of Paprika	.5	-	-	-	-
Whey	1	1	1	1	1
Mineral Premix	2	2	2	2	2
Vitamin Premix**	2	2	2	1	1
Palm Oil	-	.5	.5	-	-
Fish Oil	3	-	-	1	3
Vegetable Oil	2	-	-	-	2
Hard Fat	-	-	-	2	-
Margerine	-	7	7	-	-
Emulsifier***	-	-	-	2	-
Water	-	-	-	2	-

*To the nearest percent.

**ICN Vanderzant with dextrose replacing ascorbic acid.

***Dimidan TH monoglyceride Grinstead Products.

Table 2 Growth Analysis.

	Average initial size	Average final size	Percent increase	Average daily increase mm	% of control mm daily increase	% of control % daily increase	Mortality	% Mortality
*79-1	6.77	11.40	68	.051	75	90	18	41
S.D.	0.51	1.30						
**79-2-N	6.93	11.71	69	.053	78	92	35	80
S.D.	0.61	1.13						
*79-2-E	7.00	11.15	59	.046	68	88	23	52
S.D.	0.48	1.13						
**79-3	6.90	9.85	59	.033	45	73	13 ¹	30
S.D.	0.54	1.01						
**79-4	7.10	11.00	65	.044	59	82	7	16
S.D.	.67	1.18						
BS-1	6.80	13.40	98	.074	--	--	11 ²	25
S.D.	.62	1.14						
BS-2	6.60	12.71	92	.068	--	--	9	20
S.D.	.69	1.27						

*Diet compared to B5-2 because they were in the same water supply.

**Diet compared to B5-1 because they were in the same water supply.

¹Five Animals were missing one day when the water level went over the top of the cage.

²Six of the deaths were on the same day during a low water fluctuation.

S.D. = Standard Deviation.

Table 3 Vitamin content.

	Initial Concentration mg/100g			Final Concentration in water mg/100g			% Leached			
	Thiamine	Riboflavin	Niacin Pyridoxine	Thiamine	Riboflavin	Niacin Pyridoxine		Thiamine	Riboflavin	Niacin Pyridoxine
79-1	.844	.845	--	.574	.260	.280	.092	68	31	--
79-2-N	.966	.695	5.32	.819	.280	.247	.152	85	40	5 26
79-2-E	.579	.631	3.96	.459	.164	.174	.107	76	27	4 28
79-3	.577	.572	5.54	.582	.190	.232	.102	101	35	4 36
79-4	.641	.502	4.77	.539	.099	.260	.124	84	20	5 39

Table 4 Approximate analysis of the diets.

	Moisture	Crude protein*	Crude fat	Crude fiber	Ash	Carbohydrate	Kcal/g	Protein/calorie ratio**
79-1	25.7	20.91	6.81	2.3	4.5	39.9	3.0	.07
79-2-N	23.5	23.09	5.94	2.5	5.7	39.3	3.0	.08
79-2-E	20.8	25.07	3.38	2.7	5.9	42.2	3.0	.08
79-3	30.3	22.06	6.53	2.2	5.0	33.9	2.8	.08
79-4	29.3	21.24	4.59	2.4	5.2	37.5	2.8	.08
***Brine Shrimp	90.9	05.80	0.50	0.35	2.0	1.4	.33	.15

* 3.69 N x 6.25.

** Callagher and Brown 1975.

*** Live weight.

Table 5 Water quality

	Salinity ppt	pH	Nitrite ppm	Temperature
Tank 1	27.5	8.0	0	21.7
Tank 4	27.5	8.0	0	21.7

Table 6 Protein manipulation vs % leaching*

	Thiamine after		Riboflavin after		Niacin after		Pyridoxine after					
	Original	Leaching % lost	Original	Leaching % lost	Original	Leaching % lost	Original	Leaching % lost				
Sample 1 soft	3.46	2.30	66.5	5.21	3.40	65.2	17.29	9.54	55.2	3.20	1.96	61.2
Sample 2 dried/cubed	13.90	6.47	49.4	18.30	4.90	26.8	41.50	22.51	54.2	7.31	3.58	49.0
Sample 3 dried/film	11.44	6.58	57.5	15.20	6.02	39.6	40.90	21.11	51.6	7.74	5.00	64.4

*in mg/100g

THE EFFECT OF LOW-PROTEIN FEEDS ON THE BIOENERGETICS OF JUVENILE
LOBSTERS (HOMARUS AMERICANUS MILNE EDWARDS)

Judith M. Capuzzo

INTRODUCTION

Mass culture of commercially important omnivorous species, such as the American lobster, will be facilitated by the use of formulated feeds. The basic features of such feeds would be: (1) that the animal's nutritional requirements are met, resulting in high growth rates and no significant difference in biochemical composition from that of wild populations; (2) that the feeds are readily consumed and assimilated by the animal; and (3) that the feeds are formulated from commercially available feedstuffs, thus reducing the costs of producing the feeds and minimizing the cost of feeding in aquaculture systems.

For an adequate formulation of compounded feeds, however, an understanding of assimilation and utilization of various dietary components by an animal is needed. Protein is an essential but expensive component of an animal's diet; it is necessary for tissue growth and maintenance, but may also be catabolized as an energy source by some organisms (Cowey and Sargent, 1972; Wolvekamp and Waterman, 1960). Utilization of protein is affected by the nature of the dietary protein source, the dietary level and the ability of the organism to utilize other dietary components as sources of energy.

In previous work with the American lobster, Conklin *et al.* (1976, 1977) demonstrated the feasibility of using commercially available protein sources such as casein, egg albumin and shrimp meal in providing the protein needs of postlarval lobsters. No optimum protein level, however, has been established for postlarval lobsters. Castell and Budson (1974) have suggested that protein is utilized as the principal source of energy by the lobster, but the protein sparing action of other dietary components (lipid and carbohydrate) has not been investigated.

The objectives of this study were (1) to compare the bioenergetics of postlarval lobsters fed a brine shrimp diet with those fed less expensive formulated feeds, and (2) to investigate the relationship of dietary carbohydrate levels and protein utilization in an attempt to determine the extent to which carbohydrate might be used as an alternative source of energy by the lobster.

MATERIALS AND METHODS

Postlarval lobsters (Homarus americanus) were divided into four groups of 100 animals each and maintained in flowing seawater at 20-22°C on one of three formulated feeds or frozen brine shrimp Artemia salina (ration = 5% DW/WW per day). The formulated feeds were shrimp meal based feeds (Conklin *et al.*, 1976)

varying in both protein content (16.6-23.3%) and carbohydrate content (22.9-31.3%) and the protein:carbohydrate ratio (0.5-1.0). Growth rates, assimilation rates, respiration rates, ammonia excretion rates, O:N ratios and protein efficiency ratios of postlarval lobsters fed the formulated feeds were compared with lobsters fed brine shrimp (51% protein, protein:carbohydrate = 5.1).

Molting frequency and length and wet weight after each molt were monitored for the four groups of lobsters for the experimental period of 120 days. Food consumption rates and assimilation rates were determined for each postlarval stage from each group by direct gravimetric analysis.

Respiration rates and ammonia excretion rates of postlarval lobsters were measured at biweekly intervals during the experimental period. Respiration rates of individual lobsters were measured at 20-22°C using a Gilson Differential Respirometer; oxygen uptake was measured immediately after feeding and after a 24 hour starvation period to approximate the active and routine rates of respiration. At the end of each set of oxygen uptake measurements, the seawater in the respirometer flasks was analyzed for $\text{NH}_4^+\text{-N}$ by the method of Solorzano (1969) in order that an in situ estimate of ammonia excretion rates and O:N ratios could be made.

Each feed was analyzed for caloric content and the relative percentages of protein, carbohydrate, lipid and ash. Caloric content was measured using a Philipson Oxygen Microbomb Calorimeter. Protein, carbohydrate and ash content were determined according to the methods described by Raymont *et al.* (1964); lipid content was analyzed according to the method described by March and Weinstein (1966).

Food conversion ratios (FCR) and protein efficiency ratios (PER) were determined for each group of lobsters and significant differences were determined by analysis of variance (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The composition and nutrient analysis of the four feeds are presented in Tables 1 and 2, respectively. Protein content of the four feeds were significantly different from one another ($P < 0.01$) and thus the feed formulations provide a basis for comparison of protein utilization in lobsters fed the test feeds.

Growth of postlarval lobsters from the four experimental groups is presented in Table 3. There was no significant difference in molting frequency among the four groups. The best growth was measured among lobsters maintained on feeds A and D, followed by feeds B and C; there was no significant difference between groups A and D or between groups B and C. The best fit slopes of wet weight increases (weight increase/day) of each group are comparable to results obtained by Conklin *et al.* (1976) in a 90-day feeding trial of postlarval lobsters fed live brine shrimp (0.015 g/day) but are higher than results obtained in the same experiment with lobsters fed the shrimp meal feed (0.007 g/day).

Food consumption rates of groups B and C were significantly lower than the rates

measured for groups A and D and might explain the differences in growth rate. The formulated feeds were slightly lower in caloric content than brine shrimp and the pellets were fragmented by the lobsters during feeding, particularly pellets of feeds B and C. Assimilation rates measured in the four groups were not significantly different from one another and were equal to ~90% of the food consumed. Food conversion ratios (grams dry weight fed/grams wet weight gain) ranged from 1.90 for lobsters fed feed A to 2.20 for lobsters fed feeds B and C (Table 3).

The results of respiration rate measurements are presented in Fig. 1. Respiration rates measured immediately after feeding were highest among the lobsters fed brine shrimp and were ~37% higher than the standard respiration rate measured 24 hours later. The increased rate of oxygen consumption associated with feeding is termed the specific dynamic action (SDA) and reflects the calorogenic effect of protein catabolism. The values for SDA from lobsters fed the three formulated feeds were not significantly different from one another and were ~17% higher than the standard respiration rate. There was no significant difference in standard respiration rates of lobsters from the four test groups.

Ammonia excretion rates of lobsters from the four groups are presented in Fig. 2; excretion rates measured in each of the four groups were significantly different from one another ($P < 0.01$) and were directly correlated with the protein level of each feed.

The values for SDA, the O:N ratio (atomic ratio of oxygen consumed to $\text{NH}_4^+\text{-N}$ excreted) and the protein efficiency ratio (grams wet weight gain/grams dry weight protein fed) for each group of lobsters are presented in Table 4. The O:N ratios and protein efficiency ratios measured in the four experimental groups were significantly different from one another ($P < 0.01$) and were inversely correlated with the protein level of each feed. For lobsters fed brine shrimp, an O:N ratio of 12.9 was measured, indicating a high dependency on protein catabolism as an energy source; the protein efficiency ratio of this group of lobsters was 0.9. For lobsters fed the three formulated feeds, the O:N ratio ranged from 16.2 to 23.3 and the protein efficiency ratio ranged from 2.2 to 2.7. The increase in these parameters with decreasing protein levels in the formulated feeds provides a strong indication of the increased dependency on dietary carbohydrate as an energy source in postlarval lobsters.

To maximize both growth and protein efficiency ratios in the American lobster, energy sources in addition to protein must be utilized. Energy production from protein oxidation is both nutritionally and economically wasteful and the protein sparing action of other dietary components must be fully investigated. The results of this study are indicative that dietary carbohydrate levels induce a protein sparing action in the American lobster. Further identification of the responses of the lobster to dietary carbohydrate levels and sources is needed before feeds with optimum protein:carbohydrate ratios and protein:energy ratios can be formulated. The findings reported in this study are a preliminary framework from which this problem can be further explored.

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Table 1 Composition of test feeds (% dry weight basis).

Ingredient	Feeds			
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>
Herring meal ¹	7.77	7.77	7.77	-
Shrimp meal ²	30.58	26.21	21.84	-
Sweet whey ³	4.85	4.85	4.85	-
Soybean meal ⁴	2.91	2.91	2.91	-
Rice bran ⁵	19.42	19.42	19.42	-
Corn starch ⁴	8.74	13.11	17.48	-
Brewer's yeast ⁶	11.65	11.65	11.65	-
Vitamin mix ^{4,7}	1.94	1.94	1.94	-
Lecithin ⁴	0.97	0.97	0.97	-
Cod liver oil ⁴	4.85	4.85	4.85	-
Bernhart-Tomarelli salt mix ⁴	2.91	2.91	2.91	-
Kelgin ⁸	1.94	1.94	1.94	-
Sodium metaphosphate ⁹	1.46	1.46	1.46	-
Frozen brine shrimp ¹⁰	-	-	-	100.00

¹James Farrell & Co., Seattle

²Southland Canning & Packing Co., New Orleans

³Kraft Foods, Chicago

⁴ICN Pharmaceutical, Inc., Cleveland

⁵Uncle Ben's, Inc., Houston

⁶Millibrew, Inc., Juneau, Wisconsin

⁷Castell and Budson (1974)

⁸Kelco Co., San Diego

⁹Fisher Scientific

¹⁰Metaframe Co., Newark, California

Table 2 Nutrient analysis of test feeds¹.

Component	Feeds			
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>
% Lipid	7.75 (0.25)	7.75 (0.25)	7.75 (0.25)	8.25 (0.25)
% Protein	23.30 (0.50)	19.97 (0.50)	16.65 (0.30)	51.00 (0.50)
% Carbohydrate	22.85 (0.50)	27.47 (0.50)	31.27 (0.50)	9.98 (0.20)
% Ash	21.71 (0.50)	21.50 (0.50)	19.87 (1.00)	17.40 (1.00)
Protein:Carbohydrate	1.02	0.73	0.53	5.11
Calories/mg	2.75	2.75	2.75	3.12

¹All values are mean values of three replicate assays (\pm 1 standard error);

% dry weight basis.

Table 3 Growth and food conversion of postlarval lobsters fed the test feeds for 120 days.

Measurement	Group			
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>
Initial weight (grams)	0.189 (0.005)	0.193 (0.007)	0.192 (0.009)	0.195 (0.010)
Final weight ¹ (grams)	2.572 (0.075)	2.002 (0.110)	1.977 (0.025)	2.487 (0.113)
Weight increase/day (grams/day)	0.020	0.015	0.015	0.019
No. Molts	6	6	6	6
% Mortality	3.0	8.0	10.0	18.0
Food given ² (grams)	4.530	3.925	3.950	4.785
FCR ³	1.90	2.20	2.20	2.10

¹Wet weight, mean of 20 animals (\pm 1 standard error).

²Sum of average feeding rates of each postlarval stage.

³Food conversion ration = $\frac{\text{total dry wt. of food fed (grams)}}{\text{total wet wt. gain (grams)}}$

Table 4 Values for SDA, O:N ratio and protein efficiency ratio of postlarval lobsters fed the test feeds¹.

Measurement	Group			
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>
SDA - % ²	17.5 (1.0)	17.1 (0.6)	17.6 (0.5)	36.8 (1.5)
O:N ratio	16.2 (0.5)	17.3 (1.4)	23.3 (1.7)	12.9 (0.2)
PER ³	2.2 (0.1)	2.3 (0.1)	2.7 (0.1)	0.9 (0.1)

¹All values are mean values of stage VI through stage XI lobsters (\pm standard error).

²Specific dynamic action.

³Protein efficiency ratio = $\frac{\text{wet wt. gain (grams)}}{\text{dry wt. protein fed (grams)}}$.

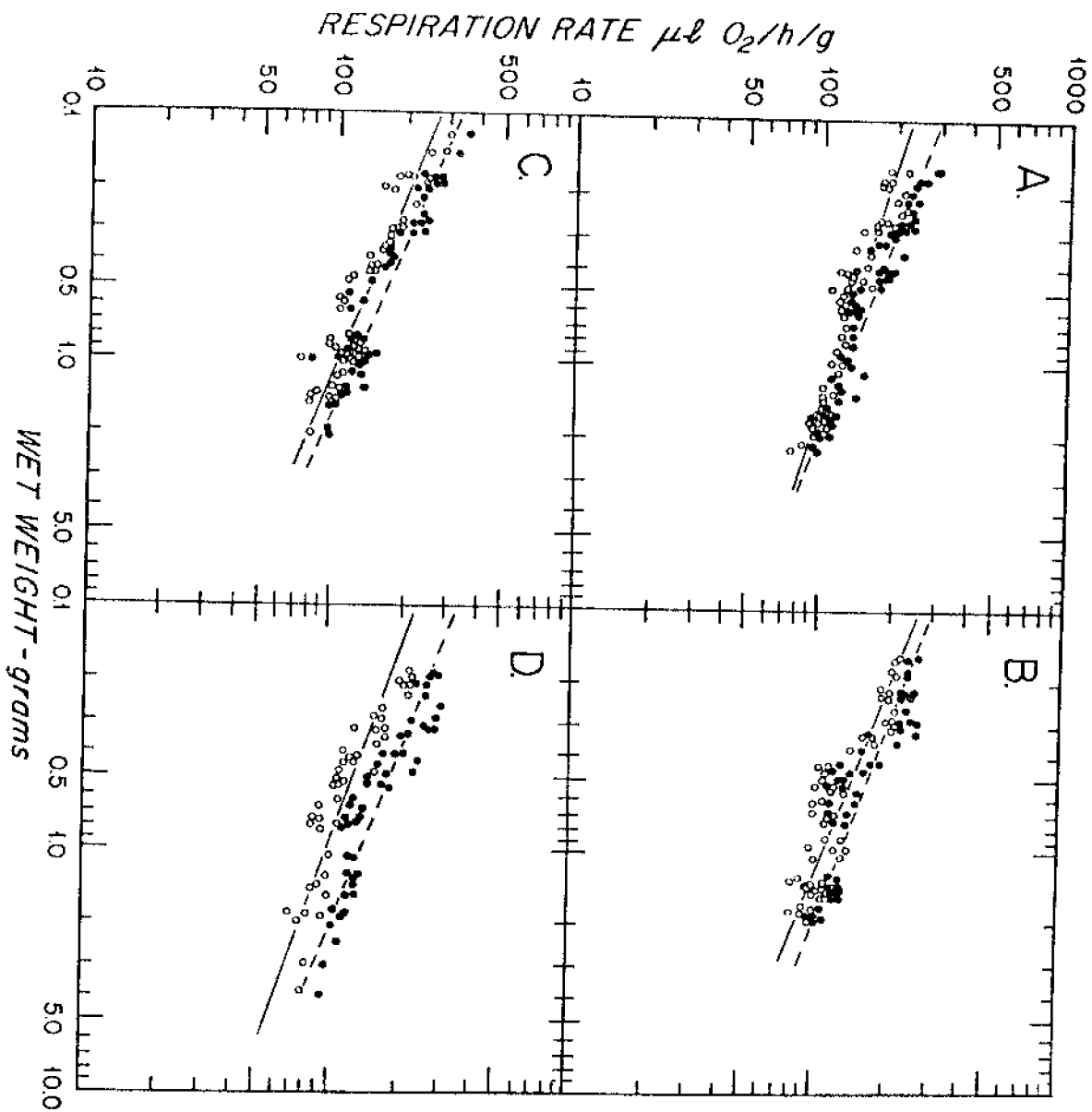


Figure 1. Log-log plots of respiration rates and wet weight of postlarval lobsters from the four test groups; closed circles, measurements made after feeding; open circles, measurements made after 24 h starvation; - - - - regression line for fed animals; ——— regression line for starved animals; A, B, C and D refer to postlarval lobsters fed the specified feeds.

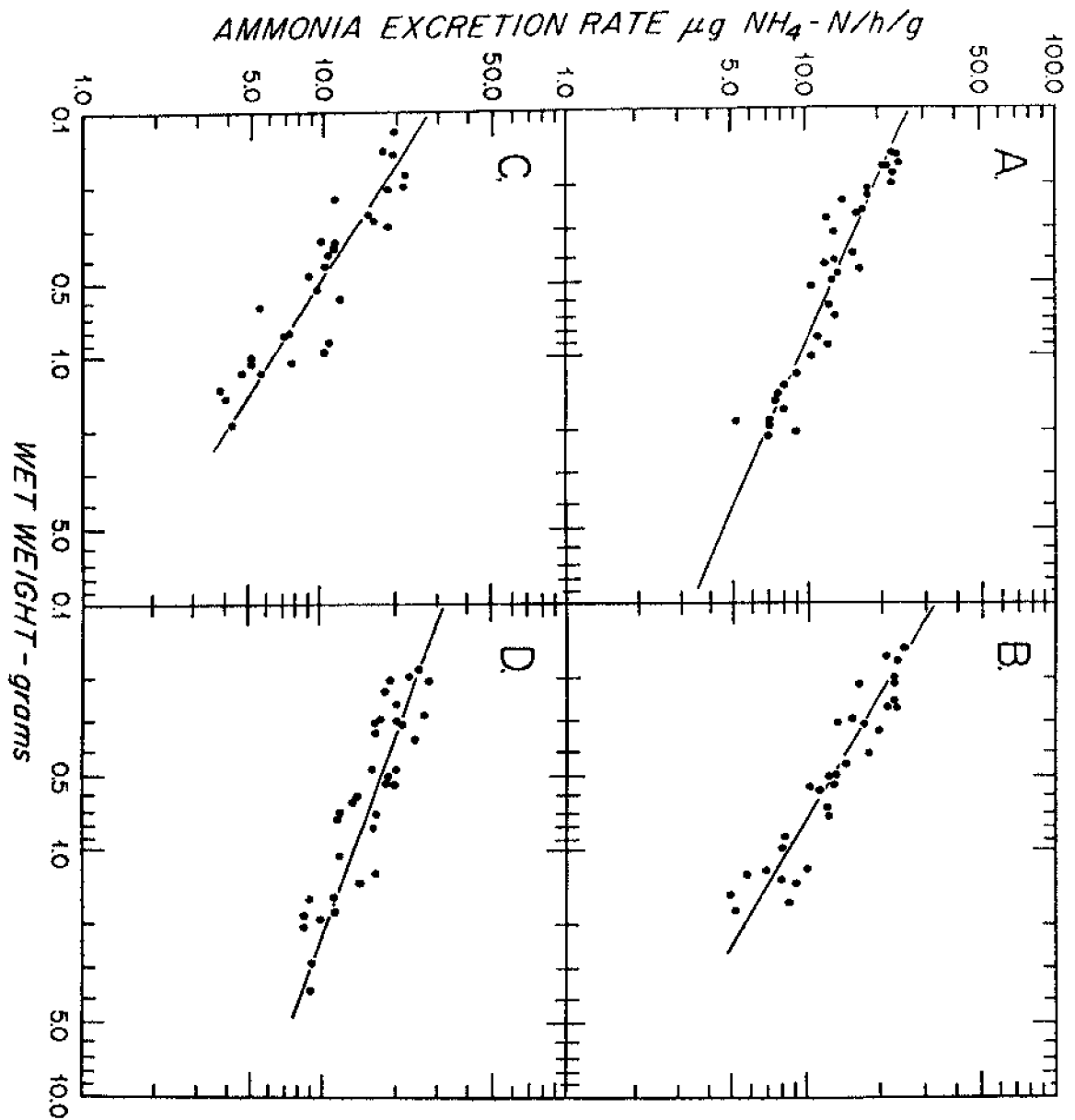


Figure 2. Log-log plots of ammonia excretion rates and wet weight of postlarval lobsters from the four test groups; ——— regression line; A, B, C and D refer to postlarval lobsters fed the specified feeds.

CONSIDERATIONS OF THE LECITHIN AND PROTEIN REQUIREMENTS OF
JUVENILE LOBSTERS (HOMARUS AMERICANUS).

Andrew Boghen and John D. Castell

INTRODUCTION

A major obstacle for the establishment of a viable commercial lobster aquaculture program remains the identification of a suitable artificial diet. One of the main ingredients of such a diet is protein and because of its universally high demand, any prospective protein source will not only have to be optimally balanced in essential amino acids but relatively accessible and low in cost.

Castell and Budson (1974) have indicated that lobsters displayed a high protein requirement (40-60% dry weight) when casein was used as the only dietary protein. Despite the necessity for such high protein levels, casein was believed to be a useful reference protein and was consequently included in a series of test diets (Castell et al., 1974, 1975, 1976). More recently casein was found to be limiting in certain essential amino acids for lobsters (Mason and Castell, 1980). In addition, the quality of casein has fluctuated in the last few years and this discrepancy in amino acid content has been confirmed through analyses performed in our laboratories and those of the suppliers (ICN, Nutritional Biochemical Corp., Cleveland, Ohio).

A recently completed study (Boghen and Castell, 1980) suggested that protein extracted from the shrimp Penaeus aztecus (Hercules Inc., Wilmington, Delaware) produced superior results when compared to casein or to protein originating from whole egg and chicken feathers. Production of the shrimp protein was discontinued, however, and attempts to substitute this with protein extracted from the shrimp Pandalus borealis failed (Castell and Boghen, 1979).

In a continuing effort to identify a useful standard artificial diet for juvenile lobsters we decided to evaluate the impact of diet H-440, a common reference diet employed in nutrition work for a large variety of finfish, including trout, salmon and catfish (NRC 1973) as recommended by Dr. J. E. Halver, University of Washington (Personal Communication).

In a different context, Dr. Douglas Conklin of the Bodega Marine Laboratory, Bodega Bay, Calif. reported good success with a lecithin supplemented diet which contained casein and albumin as the major proteins (Personal Communication).

A preliminary study was conducted to compare diets H-440, Conklin's casein-albumin diet supplemented with lecithin (diet 79II-5), the same diet without lecithin (diet 79II-2), our original casein diet and a mixed protein diet consisting of equal proportions of egg albumin, casein and shrimp.

Based partly on the findings originating from the above inquiry, a second study was initiated in which the diets containing one of the following semi-purified

proteins were tested:

1. Casein
2. Casein supplemented with Lecithin
3. Conklin's 79II-5 diet consisting of casein and albumin
4. Sea Urchin (*S. drobachiensis*)
5. Mussel (*M. peulis*)
6. Crab (*C. irroratus*)
7. Shrimp (*Penaeus* sp.)
8. Whole frozen adult brine shrimp (*A. salina*)

The significance of the findings of this study are currently being analyzed and will therefore be discussed in a general manner. A detailed paper will be presented at the 11th Annual Meeting of the World Mariculture Conference, New Orleans, LA (1980).

MATERIALS AND METHODS

Lobster larvae were hatched from egg-bearing females and raised to 4th and 5th stage juveniles at 20°C according to the method previously described by Castell (1977). For both experiments, lobsters were randomly distributed among 5 to 8 diet groups (40 lobsters/group) and raised in the recirculating artificial sea water system described by Boghen and Castell (1979).

Methods used in the preparation of the synthetic diets and for the purification of the test proteins have previously been described in detail by Boghen and Castell (1979, 1980).

The reference for the compositions of the test diets in Experiment 1 are:

1. H-440 (NRC, 1973)
2. Conklin's casein-albumin diet with lecithin (Table 1)
3. Conklin's casein-albumin without lecithin (Table 1)
4. Casein (Table 1)
5. Casein; egg albumin; shrimp (Similar to the formulation described by Castell and Budson, 1974, except that the protein fraction consists of three ingredients equally divided to total 50% of the dry diet.)

All the test diets which were studied in experiment 2 are presented in Table 1.

RESULTS AND DISCUSSION

Experiment 1

For experiment 1, the effects of the diets on percent survival and Normalized Biomass Increase (mg/wk) are presented in Figures 1 and 2.

Our findings clearly indicate that all the diets, with the exception of Conklin's

lecithin supplemented diet, were unsatisfactory. High mortalities (Figure 1) for lobsters fed H-440, casein, mixed protein and Conklin's diet without lecithin were observed by the third week. Consequently, it was to be expected that weight gain and thus an increase in Normalized Biomass would only be observed for the lecithin supplemented diet (Figure 2). Such findings lent support to the possibility that lecithin might indeed render some beneficial effects.

Experiment 2

While in experiment 2, the potential impact of lecithin was reconsidered, the primary objective of our work was to continue with our efforts in trying to identify an appropriate reference protein which could ultimately be incorporated into a standard juvenile diet.

Growth and survival data are presented in Table 2. Analysis of experimental variance using Duncan's test for significant differences at the 5% level (Steel and Torrie, 1960) suggests that the mean weight gain of crab-fed lobsters is significantly higher ($P < 0.05$) than that for animals on all other diets except diets 4 (casein supplemented with lecithin) and 8 (shrimp). See Table 3. The much lower survival rates ($P < 0.01$) of animals fed the latter diets support the probability that the diet containing the crab protein is superior (Table 2). It is important to mention that the lobsters fed diets 2 and 4 (i.e. the casein and Conklin's lecithin supplemented diets) survived well (92.5% and 90%) until about the 6th week, when they suddenly began dying in large numbers.

Preliminary analysis suggest that there is considerable fluctuation in protein purity, and that in general the marine proteins were lower in protein and higher in ash content than those diets consisting of casein and albumin (Table 4). Finally, amino acid analysis of our protein samples failed to show evidence that the amino acid content was linked to the success or failure of any given test protein.

Conclusions

- a) Our findings are consistent with other workers who have shown that beneficial effects can be obtained by using marine invertebrate proteins.
- b) There seems to be a distinct advantage to including lecithin in artificial diets for juvenile lobsters.
- c) Casein definitely appears to be deficient.
- d) Lobsters fed crab protein grew fastest.
- e) No definite inference can be made between lobster development and the amino acid content of the different proteins used for this study.
- f) Considering the apparent lower purity of our marine proteins, it is highly significant to be able to conclude that if a suitable protein such as crab is available, the optimal protein concentration required in an artificial diet may be considerably lower than that which was previously believed to be necessary (Castell and Budson, 1974).

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Table 1 Percent composition of diets 1-8 (dry weight basis) as tested in experiment 2.

Ingredients ^a	1	2	3	4	5	6	7	8
	Brine Shrimp	Conklin's ^a Casein (31.0) Albumin (4.0)	Casein (50.0)	Casein- ^d (50.0)	Sea Urchin ^e (50.0)	Mussel (50.0)	Crab (50.0)	Shrimp (50.0)
Gelatin			10.0	10.0	10.0	10.0	10.0	10.0
Gluten	15.0							
α-Cellulose	4.0		18.8	11.3	11.3	11.3	11.3	11.3
Cornstarch	26.7		5.0	5.0	5.0	5.0	5.0	5.0
Mineral Mix ^b	3.0		3.0	3.0	3.0	3.0	3.0	3.0
Cholesterol	0.5		1.0	1.0	1.0	1.0	1.0	1.0
Vitamin Mix	2.0		2.0	2.0	2.0	2.0	2.0	2.0
Cod Liver Oil			9.0	9.0	9.0	9.0	9.0	9.0
Lipid Mix S	6.0							
A/D ₃	0.1							
Vitamin E ^c	0.2		0.2	0.2	0.2	0.2	0.2	0.2
Lecithin	7.5			7.5	7.5	7.5	7.5	7.5
Choline chloride (70%)			1.0	1.0	1.0	1.0	1.0	1.0

^aAll ingredients were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio except Vitamins E, A and D, which were purchased from Roche Chemical Division, Nutley, N.J. and cholesterol which was obtained from Sigma Chemicals, St. Louis, Missouri.

^bBernhart - Tormarelli salt mix

^cConcentration of Vitamin E in diet 2 is diluted by 50%.

^dThe sea urchins were collected from the Bay of Fundy while the mussel and crab were removed from the Northumberland Strait. Frozen Penaeus shrimp tails were received from Demerico Corp., Brownsville, Texas.

^eConklin's Casein-Albumin diet without lecithin was readjusted to contain 11.5 cellulose instead of the 4% used for experiment 2.

Table 2 Growth and survival data for diets 1-8 for experiment 1.

Diet	Protein	Initial Wet Weight (mg) mean ± S.D.	Final Wet Weight (mg) mean ± S.D.	% Weight Gain	% Survival
1	-(brine shrimp)	62 ± 21	175 ± 58	182	87.1 ^A
2	Conklin's Casein-Albumin	61 ± 19	263 ± 82	323	30.6 ^B
3	Casein	70 ± 25	143 ± 94	104	13.1 ^{BC}
4	Casein-	70 ± 25	314 ± 29	350	7.5 ^C
5	Sea Urchin	70 ± 25	200 ± 59	187	87.5 ^A
6	Mussel	53 ± 16	214 ± 94	390	65.8 ^A
7	Crab	63 ± 22	343 ± 118	446	95.0 ^A
8	Shrimp	81 ± 27	297 ± 10	266	5.2 ^C

^aSignificant differences at .01% level is indicated by different superscripts.

Table 3 Analysis of variance for differences in mean weight gain of lobsters fed diets 1-8. The diet groups are arranged in increasing rank order.

Diet	Diet							
	3	1	5	6	8	2	4	7
	Casein (90.00)	Br. Shrimp (112.06)	Urchin (126.54)	Mussel (157.35)	Shrimp (172.00)	Casein-Alb. (202.64)	Casein* (250.67)	Crab (255.37)
5 Casein (90.00) ^a	--							
1 Br. Shrimp (112.06)	22.06 ^b	--						
5 Urchin (126.54)	36.54	14.48	--					
6 Mussel (157.35)	67.35	45.29*	30.81	--				
8 Shrimp (172.00)	82.00	59.94	45.46	14.65	--			
2 Casein-Alb. (202.64)	112.64*	90.58*	76.10*	45.29	34.64	--		
4 Casein* (250.67)	140.64*	118.61*	104.13*	73.32	58.67	28.02	--	
7 Crab (255.37)	165.37*	143.31*	128.83*	98.03*	83.37	52.73*	24.70	--

^aNumbers in parenthesis represent the mean weight gain after 11 weeks

^bNumbers in the table represent differences in mean weight between diet groups.

* Significant difference (P<0.05) in mean weight gain between diet groups.

Table 4 Percent ash, moisture and purity of dietary proteins.

Component	Protein					
	Casein	Albumin	Shrimp	Crab	Urchin	Mussel
ash ^a	0.9±.7	2.5±0.3	4.9±0.1	15.9±0.5	26.1±0.5	6.8±0.3
moisture ^b	9.9±.1	7.4±0.3	7.9±0.5	3.5±0.1	9.3±0.2	10.3±0.8
protein ^c	91.5	93.2	68.9	32.8	28.2	44.3

^a% dry weight basis

^bwater content after lyophilization

^c% purity based on total protein sample (g/100 sample)

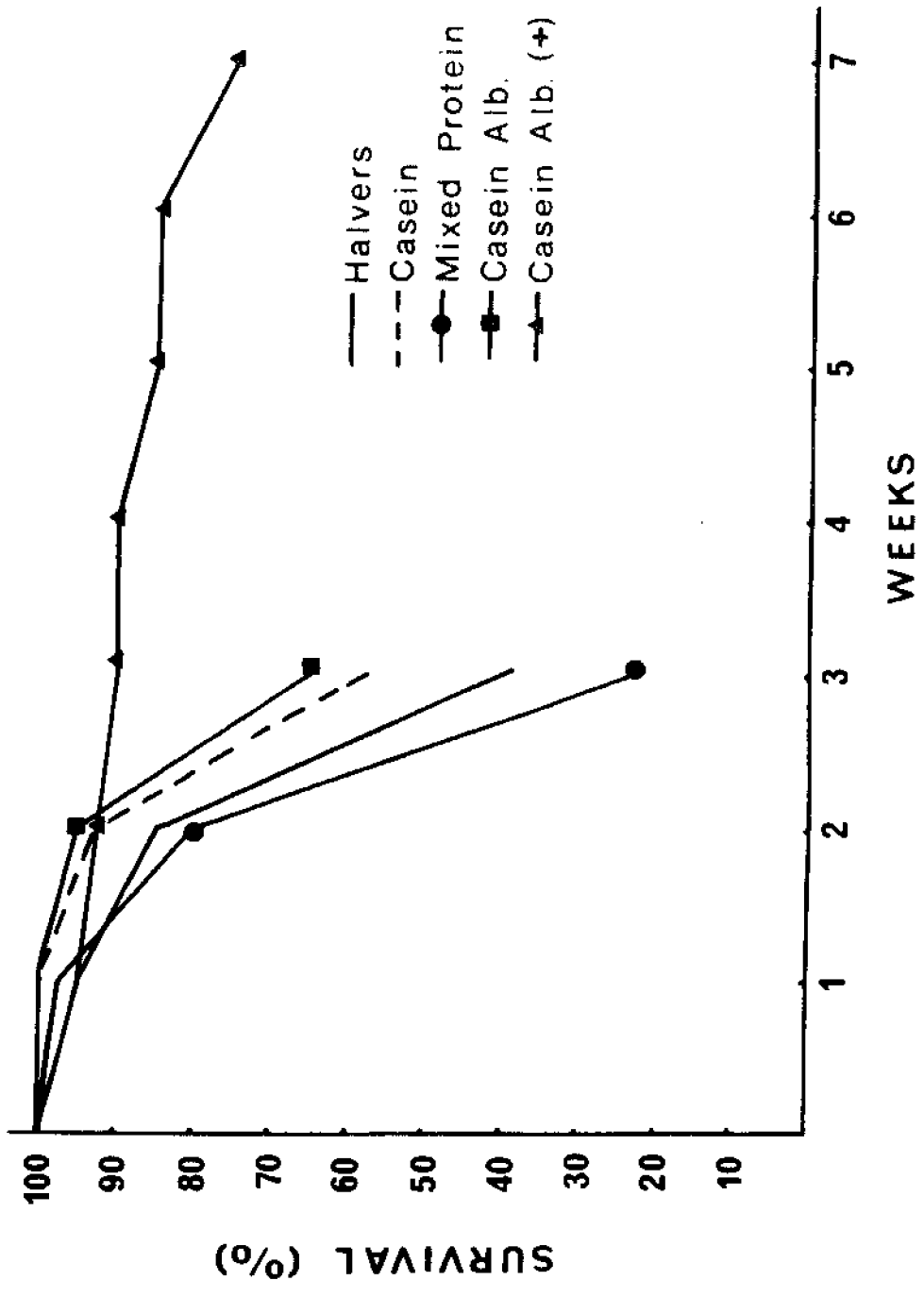


Figure 1. Percent survival of juvenile lobsters receiving different semi-purified diets in Experiment 1.

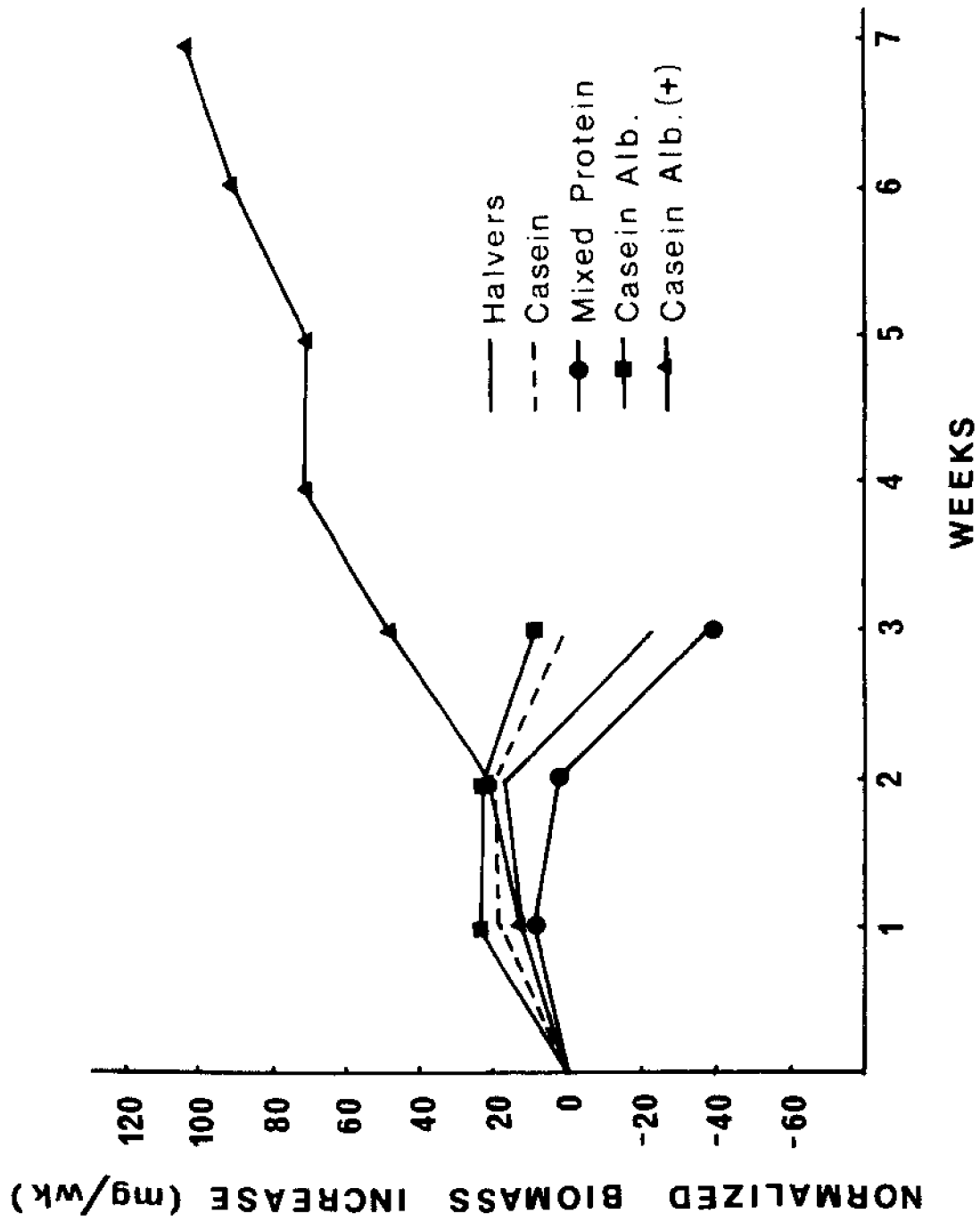


Figure 2. Normalized Biomass Increase of juvenile lobsters receiving different semi-purified diets in Experiment 1.

RECENT PROGRESS IN LOBSTER NUTRITION AT THE BODEGA MARINE LABORATORY

D.E. Conklin

Our studies on the nutrition of juvenile lobsters have indicated that the use of purified test diets resulted in high mortality rates during the first 30 days of juvenile growth unless a supplement of "natural" foodstuffs (chopped mussel tissue, chopped beef liver, live brine shrimp, etc.) was provided in addition to the regular diet (Conklin *et al.*, 1978). Recently, we found the addition of a soy lecithin fraction to the purified diet (Table 1) also served to prevent these mortalities. The optimum level of soy lecithin inclusion was approximately 8% (Table 2). A number of possible roles for lecithin in the lobster's diet have been suggested, but its exact function(s) is not understood at this time. Utilizing this diet, we have been able to demonstrate for the first time a significant response, in terms of juvenile lobster growth rates, to varying levels of our standard vitamin mixture in the purified test diets (see Fig. 1). Preliminary experiments to determine the requirement of individual vitamins within the vitamin mixture indicate there is a dietary requirement for thiamin and riboflavin.

ACKNOWLEDGMENTS

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Table 1. Components of purified diets 78S and 79F and sources of ingredients (from Conklin, et al., 1980).

Ingredient	% Dry Weight		Source
	78S	79F	
Vitamin-free Casein	31.0	31.0	1
Wheat Gluten	15.0	15.0	1
Spray-dried Egg White	4.0	4.0	1
Lipid Mix S	6.0	6.0	2
Vitamins A/D ₃	0.1	0.1	3
Vitamin E 50%	0.2	0.2	4
Vitamin Mix BML-2*	2.0	4.0	1
Mineral Mix BTm	3.0	3.0	5
Cholesterol	0.5	0.5	1
Soy Lecithin	---	8.0	6
Corn Starch	36.3	26.7	1
Cellulose	<u>1.9</u>	<u>1.5</u>	1
	100.0%	100.0%	

* Vitamin Mix BML-2 contains: Thiamin mononitrate 0.5%, riboflavin 0.8%, nicotinic acid 2.6%, Ca-pantothenate 1.5%, pyridoxine HCl 0.3%, cobalamine 0.1%, folic acid 0.5%, biotin 0.1%, inositol 18%, ascorbic acid 12.5%, PABA 3%, cellulose 60%, BHA 0.1%.

1 - ICN Pharmaceuticals, Inc., Cleveland, OH 44128

2 - Contains: Cod liver oil - 66% (ICN)
 Corn oil - 33.8% (ICN)
 Ethoxyquin - 0.2% (Monsanto)

3 - Gelatin beadlets containing 650,000 I.U. Vit. A and 325,000 I.U. Vit. D₃ per gram (Roche).

4 - Roche Chemical Division, Hoffmann-LaRoche, Inc., Nutley, N.J. 07110.

5 - Mineral Mix Bernhart-Tomarelli modified (ICN).

6 - Lecithin Co., Atlanta, GA 30302. The proximate composition of F-100 lecithin as provided by the American Lecithin Co. is Ash 8%, Phosphorus 3.1%, Notrigen 1.1%, Choline 3.6%, Inositol 2.2%, Sterols 1.2%.
 lecithin as provided by the American Lecithin Co. is Ash 8%, Phosphorus 3.1%, Nitrogen 1.1%, Choline 3.6%, Inositol 2.2%, Sterols 1.2%.

Table 2 Survival characteristics of juvenile lobsters grown on diets with lecithin of various qualities and quantities and without supplementation. Results are from different experiments (from Conklin, et al., 1980).

Diet	Percent Survival		
	30 days	60 days	90 days
78S + 5% lecithin (ICN)	46	0	--
78S + 7.5% lecithin (ICN)	100	97	89
78S + 10% lecithin (ICN)	100	86	*
78S + 20% lecithin (ICN)	97	86	*
78S + 35% lecithin (ICN)	100	89	*
79E ¹	56	0	-
79E + 8% lecithin (F-100) ²	100	100	89
79E + 8% lecithin (ICN) = 79F	100	97	92

*Dietary trials discontinued after 60 days.

¹79E = 79F with lecithin replaced by cellulose.

²supplied by American Lecithin Co., Atlanta, GA 30302.

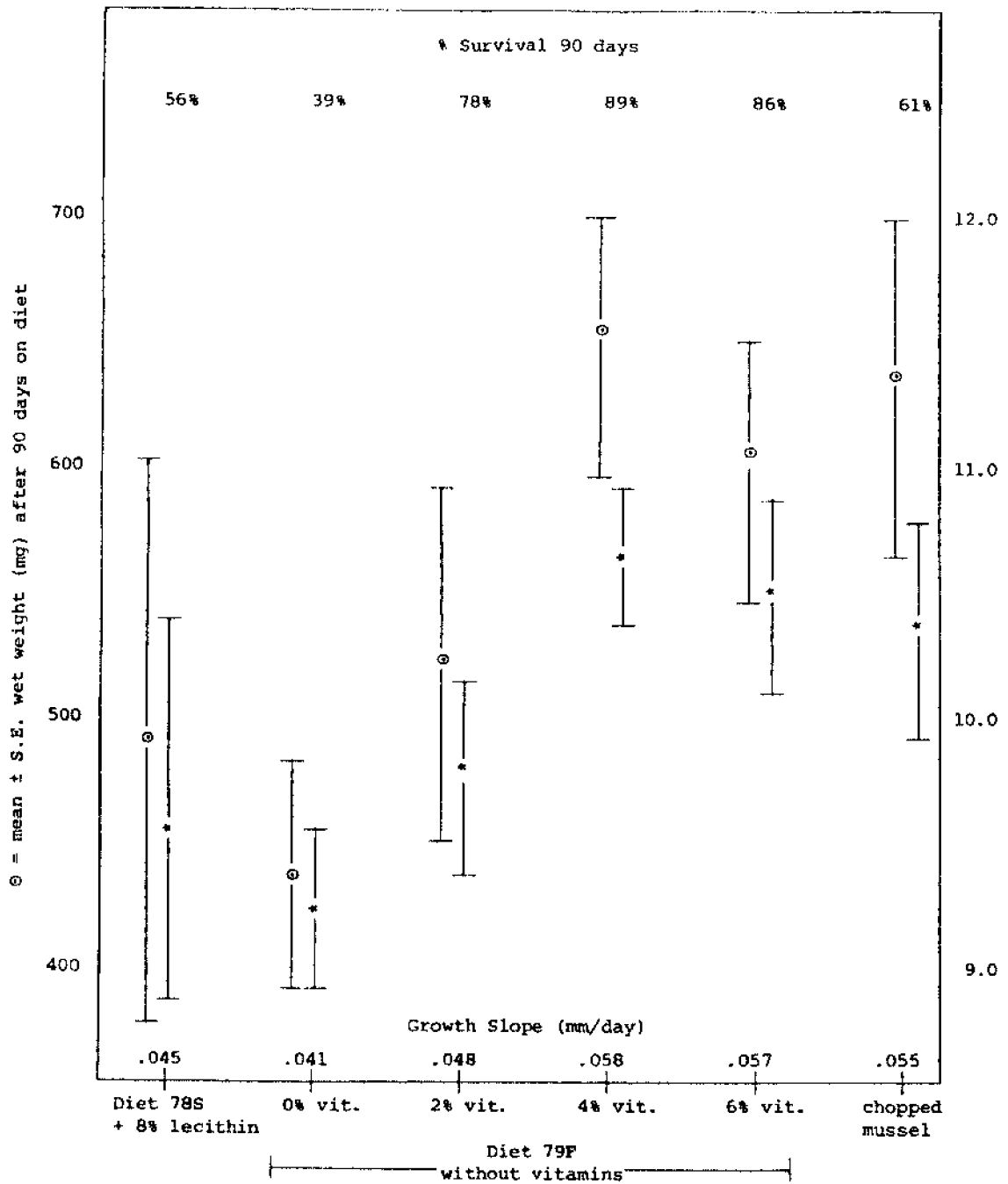


Figure 1. Survival and growth characteristics of juvenile lobsters reared on various purified diets containing lecithin and on chopped mussel.

A DIET FOR FEEDING ADULT AMERICAN LOBSTERS IN LOBSTER POUNDS

Robert C. Bayer, Margie Lee Gallagher
James H. Rittenburg and Dale F. Leavitt

INTRODUCTION

The objective of the lobster nutrition project at the University of Maine has been to develop a diet for feeding adult American lobsters held in lobster pounds. Each year in Maine and the Canadian Maritimes millions of pounds of lobsters are held in high density confinement in lobster pounds for periods of up to 6 months. A lobster pound is similar to a feed lot. Lobsters that are placed in the pound have just molted and have a small muscle mass relative to their new large and soft shell. During the period that lobsters are held in pounds they are fed in order to sustain muscle tissue growth, harden the shell and reduce cannibalism. Herring scrap from the sardine industry has been the traditional feed offered to these lobsters. Herring is variable in quality and availability; accordingly, an artificial feed of consistent composition is highly desirable.

MATERIALS AND METHODS

Lobsters were held in recirculating synthetic sea water systems or in floating wood crates in the ocean and were fed dry pelleted diets. Forty lobsters were divided into 4 groups and fed the latest diet described in Table 1 and previous diets which had .5% cholesterol replacing .5% of the flour and no kelp meal. After 10 weeks the lobsters were weighed in air and water and the weight gained was calculated. This diet has a calculated analysis shown in Table 2.

RESULTS AND DISCUSSION

The ration reported in Table 1 is presently recommended for feeding adult lobsters held captive in pounds. It differs from previous basal diets reported by Gallagher et al. 1979 in that the cholesterol has been eliminated and kelp meal added.

Surprisingly, lobsters fed this diet without cholesterol supplement gained significantly more weight, Table 3, than lobsters fed other diets. Castell et al. (1975) demonstrated that juvenile lobsters require dietary cholesterol. The diets in the present study may contain cholesterol (cholesterol analysis in progress) or other sterols from which cholesterol actually appeared to be inhibitory to weight gain.

Preliminary data with juvenile lobsters suggests that lobsters fed kelp meal at 3% of the diet may increase growth and survival, apparently without the need for

cholesterol supplementation. A fungus infection midway through the experiment caused unexpected mortalities. Because of this, the study needs to be repeated.

The diet approximates the Ca/P ratio, energy protein ratio and mineral requirements previously outlined by Gallagher et al. (1979). Components of the diet were chosen to meet specific requirements of the diet. Fish meal is present as a source of marine protein and lipids. Yeast adds protein and B vitamins to the diet. Alfalfa is a source of carotenoids. Wheat flour is used because of the binding capacity of its gluten giving the diet water stability.

The artificial feed described in Table 1 has been tested in a commercial pound containing 40,000 lobsters. No data on weight loss or gain was collected, but it was observed by a diver that the diet was consumed.

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Table 1 Basal diet for pound feeding

Fish Meal	30%
Yeast	10%
Alfalfa	10%
Flour	47%
KeIp Meal	3%

Table 2 Calculated analysis of lobster pound diet

Crude Protein	35.40%
Fat	4.90%
Ash	4.90%
Fiber	4.60%
Ca	1.09
P	.8

Ca/P = 1.33
g. protein/Kcal program = .082

Table 3 Mean air weights of lobsters (grams)

	N	Initial	Final	Gain
Cholesterol	20	508	515	04*
No Cholesterol	20	519	529	10*

Mean water weights of lobsters (grams)

	Initial	Final	Gain
Cholesterol	39	43	4
No cholesterol	36	44	8

*Anova significance at less than 0.05

SOME CURRENT FINDINGS OF THE HALIFAX LOBSTER NUTRITION GROUP

Don J. Trider and John D. Castell

INTRODUCTION

Over the past seven years our nutrition studies with juvenile lobsters have used two tray types. One type has solid plastic sides and bottom with the water entering at one end and draining at the other. The other has a mesh bottom (nylon window screen) and sits in a trough or reservoir with a continuous flow through of water. Each tray type holds twenty lobsters individually with the latter giving the animal more space. The solid bottom tray retains food material, and animals do not lose claws by sticking them through a mesh bottom. The mesh bottom tray stays cleaner as waste drops through the bottom, and animals are not as easily affected by water stoppages as there is a larger surface area for oxygen exchange and a greater reservoir of water from which the animals can obtain oxygen. Both tray types have given satisfactory results in previous studies, but no direct comparisons were made. Two diets, a natural diet and a casein diet (Table 1), were fed to juvenile lobsters (4th and 5th stage) in both tray types. Growth and survival were compared.

In a subsequent study using mesh bottom trays, casein diets varying in lecithin content were fed to lobsters. Crude soy lecithin (CSL) was fed at 0.5, 1, 2, 4, 6 and 10% levels and purified soy lecithin (PSL), crude egg lecithin (CEL) and purified egg lecithin (PEL) were fed at the 6% level. The purification consisted of sterol removal through recrystallization from methanol. Growth and survival were checked over a six week period.

RESULTS AND DISCUSSION

Comparison of Tray Types:

Survival of lobsters fed the casein diet was poor after four weeks, with almost twice as many animals surviving in the solid bottom trays (Table 2). Animals fed the natural diet had much better survival, with the solid bottom trays again giving slightly better results. These animals also had significantly higher weight gains. We stopped feeding the casein diet after four weeks but continued the natural diet for another two weeks. Survival was about 50% in both tray types at this time, but animals in the solid bottom trays had significantly higher weights. This study was terminated after six weeks due to mortalities caused by water stoppages and oxygen depletion in the solid bottom trays. This was one of the disadvantages of this type. Animals in the mesh bottom trays most likely gained less weight because food became unavailable as it dropped through the bottom.

In order to combine the advantages of both tray types, we are placing solid bottoms on the previously mesh bottom trays and putting mesh covered holes in the sides for water circulation. This tray will also sit in a trough of water to

minimize oxygen deficiencies caused by temporary stoppages in water supply.

Dietary Lecithin:

Soy lecithin had previously been shown to enhance survival in juvenile lobsters (Conklin, personal communication). In the present study, survival increased with increasing lecithin level to 4-6%, after which it remained constant to 10% (Fig. 1). Conklin found 7.5% CSL to be optimum, whereas 6% seemed to give as good survival in this study. The same trend was noted with average weights: higher weights with higher lecithin levels (Fig. 2). It appears that CSL has a factor necessary for good survival and growth of juvenile lobsters.

Lobsters fed egg lecithin had lower % survival than those fed soy lecithin, especially those fed PEL (Fig. 3). The average weight, however, was highest in the CEL fed group (Fig. 4). Since the purified forms of lecithin did not give greater weight gains or % survival it seems that some other factor in crude lecithin is also beneficial to growth and survival. We plan to do more work in this area, using a variety of phospholipids to answer these questions.

Standard Reference Diet:

It was proposed that researchers working in the field of lobster nutrition try to use a common control diet, similar to those used by Conklin and Castell, but with rock crab (*Cancer irroratus*) as the protein source. This protein could be prepared at one location and obtained from this source by each researcher or group to standardize diet trials. Nova Scotia Technical College was suggested as a possible source. In the same vein, it was also suggested that the various research groups set down guidelines to standardize the methodology used in lobster nutrition studies. An ICES (International Council for the Exploration of the Sea) Report entitled "Report of Study Group on Standardization of Methodology in Fish Nutrition," Hamburg, Mar. 21-23, 1978, was suggested as a guide in developing standard methods. Standard methods and a common control diet would facilitate comparison of results from different research groups.

Table 1 Percent composition of the natural and casein based diets.

<u>Natural Diet</u>			
Shrimp waste (heads, shells) ¹	50	Casein	40
Crab meal	15	Gelatin	10
Fish meal	20	α -Cellulose	27.8
Wheat Middlings	10	Corn starch ₃	5
Gelatin	5	Mineral mix	3
		Glucosamine	1
		Cholesterol	0.5
		Vitamins	3.2
		Cod liver oil	9.5

¹Shrimp Pandalus borealis

²Ingredients from ICN

³Modified Bernhart-Tomarelli

Table 2 Survival and average weights of lobsters fed the natural and casein diets after 4 weeks. (m=mesh bottom tray; s=solid bottom tray)

<u>Diet</u>	<u>Survival (/60)</u>	<u>Weight (mg) (ave+SD)</u>
Casein (m)	15	124 ₊₃₀
Casein (s)	27	136 ₊₃₆
Natural (m)	54	220 ₊₅₂
Natural (s)	58	256 ₊₅₀

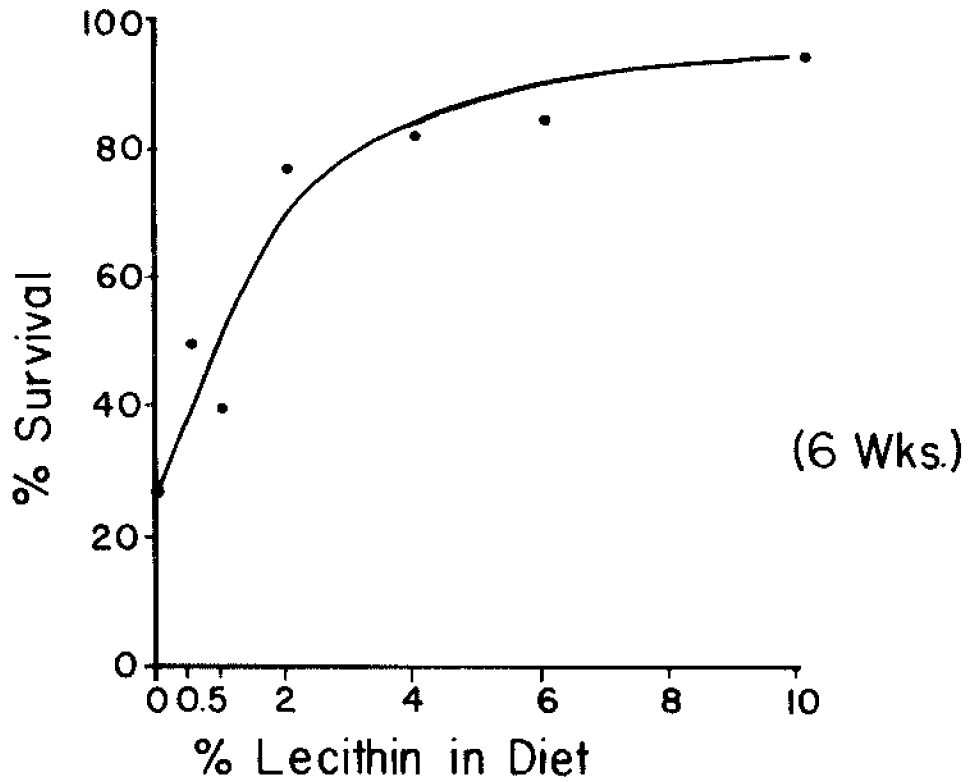


Figure 1. Survival of juvenile lobsters after six weeks feeding with various levels of lecithin.

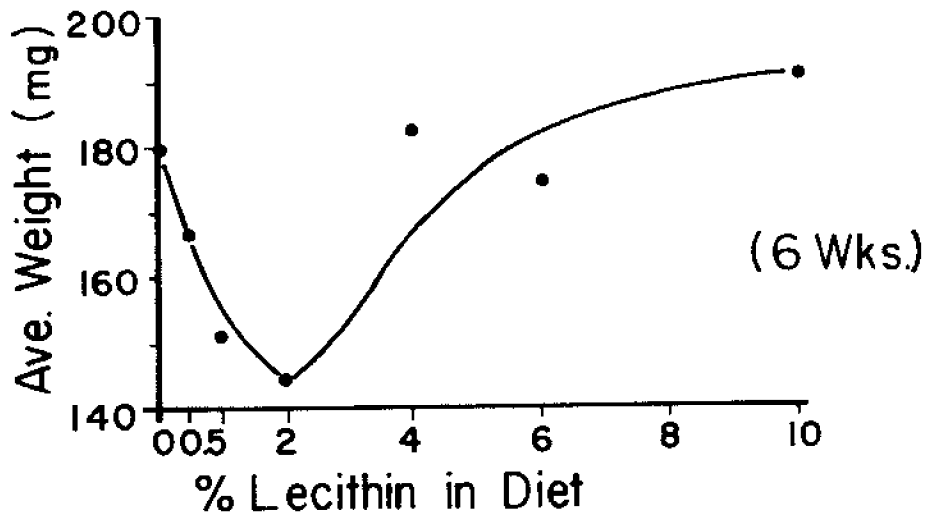


Figure 2. Average lobster weights after six weeks feeding with graded levels of lecithin.

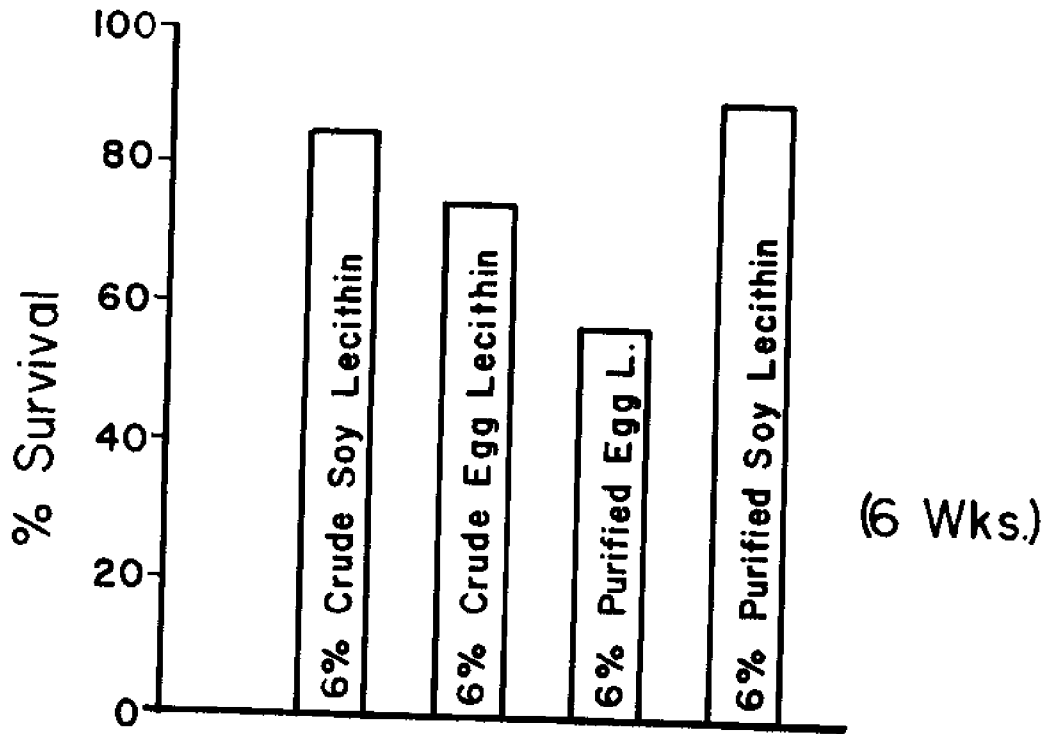


Figure 3. Comparison of lobster survival after six weeks, produced by two qualities of egg and soy lecithin.

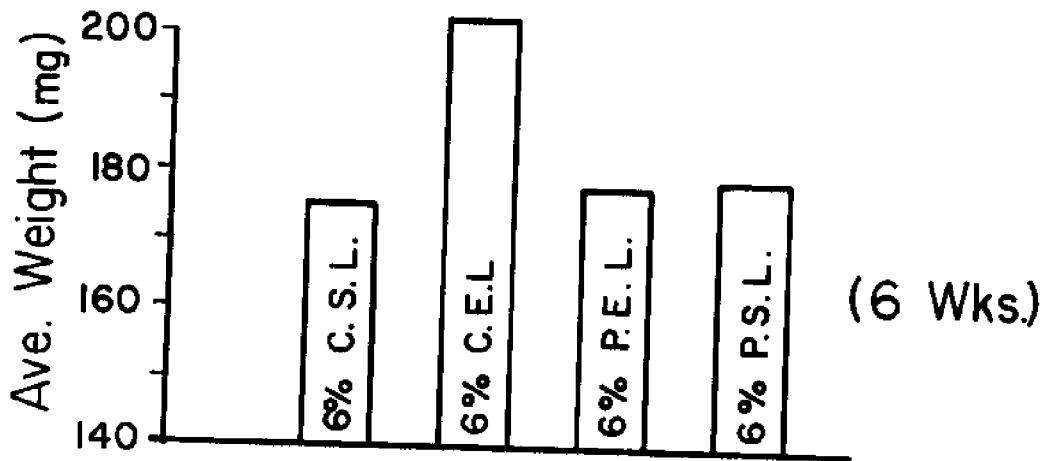


Figure 4. Average lobster weights after six weeks of feeding diets containing two qualities of egg and soy lecithin.

GROWTH AND COLOR OF JUVENILE LOBSTERS (HOMARUS AMERICANUS)
KEPT ON DIETS OF NATURAL AND ARTIFICIAL FOODSTUFF

Anthony D'Agostino

INTRODUCTION

A hatchery was established at the New York Ocean Science Laboratory for the specific purpose of producing large numbers of juvenile lobsters for the restocking of Fort Pond Bay, and to make available specimens and facilities to carry out research on those aspects of the biology of the lobster perceived as most germane to aquaculture.

Larvae were hatched from November through August and the resulting juveniles were released throughout the year except during January and February. In order to measure the probable survival of the different hatches of juveniles intended for release into the wild, appropriate numbers of animals were isolated in perforated cannisters submerged in Fort Pond Bay, as well as in laboratory troughs with running sea water. Mortality was monitored weekly. The results indicated that, by excluding predation, the survival of stages IV and VII could be expected to exceed 50% and 90% respectively.

Surprisingly, survivors, which were left in such cannisters for long periods of time unattended and without the addition of feeds, exhibited measurable growth and normal exoskeletal pigmentation. These observations stood in stark contrast to the fair to poor survival and drastic exoskeletal depigmentation shown by those control animals that were being fed a steady diet of artificially compounded rations. Apparently, the lobsters which were being kept in cannisters, and unfed, were eating the fouling organisms which settled on the inside surfaces of the cannisters, thus getting good nutrition in the process. These results prompted the design of several experiments intended specifically for the measuring of growth and development of in vivo exoskeletal pigmentation in response to various nutritional regimens.

This opportunistic follow-through program of studies was especially desirable at the time because the scarce knowledge of the trophic requirements of the lobster was, and is still, a significant obstacle in the aquaculture of this species.

MATERIALS AND METHODS

Growth in Cannisters With and Without Feeds:

Typically, juveniles of known age and size were isolated in perforated rigid plastic tubes (cannisters) of different sizes. Some were submerged in the bay, some in spillways, and others in indoor troughs with running sea water. Concurrently, an appropriate number of animals of corresponding age and size were

kept in uncapped cannisters in laboratory troughs and were fed daily ad libitum a variety of feeds according to the protocol of the experiments. The control animals were monitored daily, left over food was removed, and the rations renewed. The experimental animals were examined once per month. Data were collected on mortality, carapace length, weight and color.

Growth and Color as a Function of Feeding Natural and Artificial Foodstuff:

The typical nutritional experiment was carried out with juveniles isolated in open cages 3 x 3 x 3 inches, suspended in recycled running seawater at $15^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Usually the animals were of stage V and were grown on a particular diet for 90 to 100 days before the experiment was discontinued. On several occasions older animals were employed, in which case the size of the cages was 4 x 6 x 3 inches. The animals were monitored daily for food consumption, mortality and molting. Records were kept of the initial, intermediate and final carapace lengths, coloration and weight.

Specific information on the prevailing conditions for the experiments reported in this communication are given in footnotes to the appropriate table.

Some fragmentary information on the composition of some natural and artificial feeds that will be mentioned here is reported in Table 1.

RESULTS AND DISCUSSION

During the spring and summer months, the rate of growth of lobsters held in cannisters without the addition of feeds was nearly equal to that of the corresponding controls that were being kept in open cannisters and fed daily ad libitum (Table 2).

In the fall and early winter the growth of unfed lobsters held dockside, as well as those held in troughs in the laboratory, was less than that of the fed controls (Table 3). The size of the cannisters influenced growth, i.e. the larger the containers, the greater were the gains (Table 3). This probably was a reflection of the rate of recruitment and therefore of the availability of prey organisms rather than the effect of spatial physical factors acting directly on growth. The unfed animals, held at dockside, may have been limited by the onset of winter with its characteristically low ambient temperature and reduced fouling activity. The animals held unfed in troughs with recycled running seawater, while exposed to a nearly constant temperature, may have experienced a more severe decline in abundance and variety of fouling organisms.

Obviously, the cannisters, acting as collectors of fouling organisms, such as amphipods, crab larvae, mussels and tubicolous worms, provided the juvenile lobsters with a nutritional regimen which was qualitatively, if not quantitatively, better than any artificial feed devised to date. This suggested an approach to the culture of the lobster which, if coupled to thermal effluents of power plants and the occurrence of fouling activity sustainable throughout

the year, could prove more feasible than aquaculture in a closed system entirely dependent on artificial feeds.

Through these experiences, it became obvious that the pigmentation of the exoskeleton of the lobster varied according to the relative abundance of the species of fouling organisms trapped by the cannisters. Thus, lobsters held dockside, which had the most varied population of tubicolous amphipods, developed the darkest and most normal coloration. The lobsters held unfed in troughs with recycled running seawater were also brown, but were less dense and without the dark pigmentation of the wild type. The lobsters held in open cannisters, fed natural feeds other than amphipods, invariably were devoid of dark pigments. Lobsters fed an artificially compounded diet varied in color from gray to colorless.

The latter observations prompted the comparative study of the contribution of natural and artificial feeds on the *in vivo* development of pigmentation. In fact, the focus was shifted from studies of the nutrition of the lobster with artificially compounded ration to studies on the comparative performance of crude and artificial foodstuff.

The results of a typical experiment designed to compare the growth and coloration of juvenile lobsters maintained long-term on specific diets are reported in Table 4.

The performance of the artificial feeds was grossly inferior to either of the two natural products. The latter, in turn, supported greater growth than live or frozen *Artemia*. For example, the data shown on Table 4, recalculated according to Dr. Conklin's method for normalized biomass, give values for shrimp, amphipods and live *Artemia* of 3.29, 1.95 and 1.36 respectively. None of the artificial feeds approach these values. Surprisingly, the shrimp diet which supported the greatest survival and growth and which was nearly three times more effective than live *Artemia*, did not promote deposition of brown, green or black pigments in the exoskeleton of the lobsters. A steady diet of grass shrimp produced a deep blue exoskeleton. Only the amphipod diet permitted development of a wild type coloration.

Among the artificial feeds reported in Table 4, the Gmeiner feed #2 supported about twice the amount of growth reported for artificial feed compounded for nutritional studies at Bodega Bay, about one-half as much growth obtainable with a steady diet of *Artemia*, and only one-fifth as much growth as could be obtained with a diet of grass shrimps. Unfortunately, all information on the formulation of this feed was not published.

Useful information on the nutrition of the lobster may still be gained from the utilization of raw natural foodstuff. Currently, the data available here and elsewhere suggest that gross imbalance of utilizable protein coupled to slight deficiencies of growth factors may be responsible for the less than adequate support of long term growth by artificial feeds. With respect to the former, it may be necessary to explore the digestive and assimilation characteristics of the lobster. These may not be compatible with the so-called least-cost-formulation method of preparing feeds, which may contain a poor quality protein, undigestible in the gut of the lobster.

Table 1 Analysis of certain natural and artificial feeds¹

	Dry Wt.	Composition as Percent of Wet Wt.			
		Protein ²	Lipids ³ Total	Fatty ³ Acids	Cholesterol ⁴
Amphipods ⁵	23.5	13.2	1.16	0.824	0.19
Shrimp ⁶	21.4	12.3	1.13-1.57	0.744-1.18	0.22
Meyers' FDSC ⁷	100.0	16.9	7.02-8.10	6.17-7.25	0.48
Scott's # 2 ⁸	95.6	41.3	8.51-12.3	7.72-11.5	0.45
Gmeiner #1 ⁹	79.5	17.5	3.95-5.21	3.35-4.61	0.34
Gmeiner #2	78.3	18.8	4.68-6.75	3.97-5.18	0.32

¹The foodstuffs were analyzed in order to have a comparative measure of the gross composition of the feeds.

²Biurit, measures soluble proteins only, Raymont, et al., 1964.

³Methods in Enzymology, Colowick, S.P. and N.O. Kaplan, eds, Vol. III, pp.301-02, Academic Press, N.Y., 1957.

⁴Hank's Physiological Chemistry, Oser, B.L., ed. McGraw-Hill, N.Y., 1965.

⁵Mainly Callinectes laeviusculus collected from Fort Pond Bay, Montauk, N.Y.

⁶Palaemonetes sp. (grass shrimps) collected in Lake Montauk, N.Y.

⁷Typical artificial formulation compound for shrimps, Meyers, S.P. and Z.P. Zein-Eldin, 1972.

⁸Artificial Ration Compounded Specifically for the Lobster. Scott, M.L., et al., 1975.

⁹Artificial formulation compounded specifically for lobster. Mr. Gmeiner, no known reference in the literature.

Table 2 Survival and growth of juvenile lobsters, Homarus americanus kept with and without feeding in perforated cannisters

Conditions ¹	Mean Initial Wt. g	(N)	S	Mean Growth Days	Mean % Gain	Mean % Gain/Day
No Food Dockside	1.13	(12)	9	79	160	2.25
Fed Trough	0.43	(6)	5	77	287	3.62
No Food Trough	0.36	(3)	3	103	414	4.02
Fed Trough	0.35	(3)	3	90	336	3.72

¹The lobsters were estimated to be in stages VI-XII, were not selected for size or age, were assigned to the cannister randomly. The cannisters were 40 cm long with a diameter of 6 cm. They were maintained according to the conditions specified above. The temperature varied from 15°C to a high of 20°C. The animals receiving feeds were monitored daily while the non-fed animals were examined once a month.

(N) - Initial number of animals

(S) - Survivors

Table 3 Survival and growth of juvenile lobsters, Homarus americanus kept with and without feeding in perforated cannisters of different sizes.

Conditions ¹ Cannisters size in cm	Mean Initial Wt. g	(N)	S	Mean Growth Days	Mean % Gain	Mean % Gain/Day
No Food Dockside						
6 x 17	0.130	(5)	3	121	143	1.22
6 x 30	0.160	(4)	3	113	242	2.14
6 x 43	0.204	(4)	3	126	410	3.26
No Food Trough						
6 x 17	0.150	(6)	4	71	118	1.67
6 x 30	0.159	(3)	3	132	302	2.29
6 x 43	0.237	(4)	4	99	276	2.79
Fed Trough						
6 x 43	0.366	(13)	12	94	545	5.81

¹The lobsters were selected and assigned to the cannisters randomly. They were estimated to be in stages VI to X. They were maintained according to the condition indicated above. The cannisters were 6cm in diameter and of varying lengths. The temperature varied seasonably from 20°C to 12°C for the animals suspended dockside at Fort Pond Bay, while for those kept indoors in troughs with running seawater, the temperature regimen may have varied from a high of 25°C in late summer to a low of 14°C in early winter.

(N) - Initial number of animals.

S - Survivors.

Table 4 Survival and growth of juvenile lobsters, Homarus americanus, fed natural and artificial foodstuff¹.

Ration ²	% S	Average		Cumulative		
		# of Molts	Gain Wt. g	Gain c.l. ³ cm	Color ⁴ Change	% Gain Wet Wt.
Shrimp ²	100	4.6	3.28	1.04	-1.3	2127
Amphipods ⁶	90	3.8	1.94	0.82	+0.3	1361
Scott # 2	40	2.2	0.55	0.37	-3.3	260
Gmeiner # 1	90	3.4	0.72	0.46	-3.4	409
Gmeiner # 2	90	3.3	0.89	0.54	-3.8	489
Meyers FDSC	40	2.7	0.19	0.20	-2.8	262

¹There were 10 animals in each group, estimated to be at stage VI when selected for the experiment. They were maintained in containers 3 x 3 x 3 inches in running seawater. The temperature ranged for 15°C to 22°C during the course of the experiment which lasted 100 days.

²Rations were renewed daily.

³Carapace length.

⁴Color of carapace was scored on a scale of 1 to 5, colorless through blue to brown-green, respectively. A negative sign indicates loss of pigmentation. Conversely, a positive sign indicates a gain in pigmentation.

⁵Palaemonetes sp. (grass shrimp)

⁶Mostly Callinectes sp.

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COMPARISON OF NON-DESTRUCTIVE AND DESTRUCTIVE PARAMETERS FOR THE MEASUREMENT OF GROWTH IN ADULT AMERICAN LOBSTERS

Margie Lee Gallagher, Dale F. Leavitt
Robert C. Bayer and James H. Rittenburg

INTRODUCTION

Growth is defined as an increase in the structural and organ tissues (Maynard and Loosli, 1969). True growth should be distinguished from increases in body mass resulting from fat deposition in the reserve tissues. Growth is most commonly measured as an increase in body weight or body weight and length. However, these methods are limited because they do not demonstrate the nature of the tissue formed. Weight gain does not in all cases reflect a deposition of tissue at all, but may result from increases in water content of tissues. This type of gain is often associated with disease states in humans and other mammals. Therefore, true growth can only be measured by slaughter procedures or balance experiments which often are not feasible, are too expensive, or both. These problems are further complicated in marine animals, such as lobsters, which have discontinuous growth cycles, where large amounts of water are periodically replaced with body tissues. These problems directly affect the work in our laboratory on investigating the nutritional requirements of adult (legal size) lobsters held in pounds. Therefore, work described here was undertaken to compare several non-destructive growth parameters with procedures requiring the slaughter of the animal, in order to determine which of the non-destructive parameters best reflect true growth as indicated by dry weight and total body protein in adult lobsters.

METHODS

Wild adult lobsters with no missing appendages were obtained from the Tidal Falls Lobster Pound. Twenty-three females and 21 males were used in the study. To eliminate differences due to stage of molt, all lobsters were determined to be in intermolt stage C_4-D_0 according to Aiken (1973). Non-destructive parameters measured were carapace length using vernier calipers (measured to the nearest 0.05 mm), live weight in air using a top loading Mettler balance (measured to the nearest 0.01 g), live weight in water using a weighing chamber attached to a Mettler balance suspended in seawater (measured to the nearest 0.1 g), and volume using water displacement (measured to the nearest 1 ml). Destructive parameters measured were dry weight, total protein, and ash. Dry weight was determined by freezing and drying lobsters to a constant weight at 60°C. Total body protein was determined by the Lowry method on whole lobsters ground in a Wiley Mill through a 40 mesh sieve. Total ash was determined by ashing in a muffle furnace to constant weight at 600°C.

Standard t-tests were used to compare means of male and female lobster parameters. Non-destructive growth parameters were correlated with destructive growth parameters according to Snedecor and Cochran (1967). A t-test was used to determine the significance of correlation.

RESULTS AND DISCUSSION

There were no significant differences between the means of male and female groups with regard to live weight in air or water, volume, carapace length, dry weight, protein, or ash (Table 1). These data indicate that the lobsters in the study fell into the same population group. Legal lobsters are defined as lobsters with carapace lengths between 80-127 mm. Therefore, these lobsters were on the lower limit of legal size lobsters for catch in Maine, since the carapace lengths for these lobsters were 87.3 mm and 86.9 mm for male and female lobsters, respectively.

Table 2 presents sample correlation coefficients (r) for non-destructive growth parameters when correlated to the destructive growth parameters of dry weight and total body protein. The sample correlation coefficient is a measure of the degree of closeness in the linear relationship between two independent variables. A t-test for significance of r will determine if there is a significant linear relationship between two independent variables. As the significant r values increase, more and more of the total variance can be accounted for as linear regression, since r^2 can be described as the proportion of the variance attributed to linear regression (Snedecor and Cochran, 1967). As the r value increases, one variable becomes an increasingly better indicator of the other. Therefore, in this study, as the r value increases the non-destructive parameter becomes a better indicator of dry weight or protein, and therefore, a better reflection of true growth. From the r values in Table 2 the best correlation with dry weight for female lobsters was obtained with weight in water, where 67% (r^2) of the variance is due to linear regression. However, all other non-destructive measures were significantly correlated to dry weight and total protein for females and combined female and male data, with weight in air giving an r value of .78, when correlated to dry weight. From these data, it can be concluded that weight in air is a good estimate of true growth in female lobsters (as determined by dry weight) when compared to other more time consuming methods, such as volume or weight in water. The same is true when the non-destructive parameters are correlated to total body protein, although much less of the variation is accounted for by linear regression.

There is no significant correlation between any of the non-destructive parameters and dry weight or protein in male lobsters with the exception of weight in water. There can be several reasons for a non-significant r value. First, the data may not be linearly related, but could be related in some curvilinear fashion. In this case, the scatter plots for dry weight vs non-destructive parameters give no indication of other relationships.

Second there may not be sufficient range in the data points for any relation-

ship to be significant, since the data is clustered around one point. Since fishing pressures have caused the lobster catch to be just at the legal limit, the range of sampling for this study is reduced. However, the data for the females was significant and the scatter plots give no indication of clustering (Figure 1).

The third alternative for non-significant r value is that there is no correlation between variables or variation due to other factors is too high for the correlation to be evident. Male lobsters at this age are reaching sexual maturity, therefore variation could be due to the influence of this physiological phenomenon. The higher correlation in females would tend to support the theory that female lobsters are not sexually mature at the lower limit of the legal size (Herrick, 1895).

In summary, the data presented here indicate that all of the non-destructive parameters used to indicate growth in female adult lobsters correlate significantly with dry weight and protein, with weight in water giving the highest correlation coefficient. There were no significant correlations between non-destructive and destructive parameters for male lobsters with the exception of weight in water vs dry weight. This phenomenon may be due to onset of sexual maturity in these lobsters. It would be interesting to look at these parameters in juvenile lobsters where growth is faster and onset of sexual maturity would not be a factor.

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Table 1 Comparison of measured parameters of male and female adult lobster populations

Parameters	MALES		t-value
	Mean \pm SD (g)	Range (g)	
Weight in Air (g)	531.3 \pm 94.1	406.8-791.8	
Weight in H ₂ O (g)	45.9 \pm 5.3	35.3- 59.9	
Volume (ml)	481 \pm 92	363 -726	
Carapace Length (mm)	87.33 \pm 4.80	80.80- 96.9	
Dry weight (g)	144.5 \pm 16.9	110.9-185.4	
Protein	43.6 \pm 8.8	25.0- 60.1	
Ash (g)	43.4 \pm 4.8	30.1- 53.2	

Parameters	FEMALES		t-value
	Mean \pm SD (g)	Range (g)	
Weight in Air (g)	527.4 \pm 117.6	413.9-903.8	0.12NS
Weight in H ₂ O (g)	45.2 \pm 11.2	33.6- 76.1	0.28NS
Volume (ml)	473 \pm 112	371 -845	0.28NS
Carapace Length (mm)	86.9 \pm 6.40	81.10-107.65	0.25NS
Dry weight (g)	142.5 \pm 33.2	105.1 -247.0	0.25NS
Protein (g)	47.3 \pm 14.9	30.7- 87.6	0.98NS
Ash (g)	41.9 \pm 7.6	33.4- 57.4	0.77NS

Table 2 Sample correlation coefficients of non-destructive growth measurements with dry weight and protein content in the american lobster.

Parameter	Dry Weight			Protein		
	male	female	combined	male	female	combined
Air wet weight	0.34 NS	0.78*	0.65*	0.23 NS	0.57*	0.45*
Water wet weight	0.57*	0.82*	0.78*	0.25 NS	0.57*	0.49*
Volume	0.30 NS	0.75*	0.61*	0.21 NS	0.55*	0.43*
Carapace length	0.34 NS	0.73*	0.62*	0.07 NS	0.47*	0.34*

*Indicates significant t-test for correlation at 95% level.

NS Indicates non-significant t-test for correlation at 95% level.

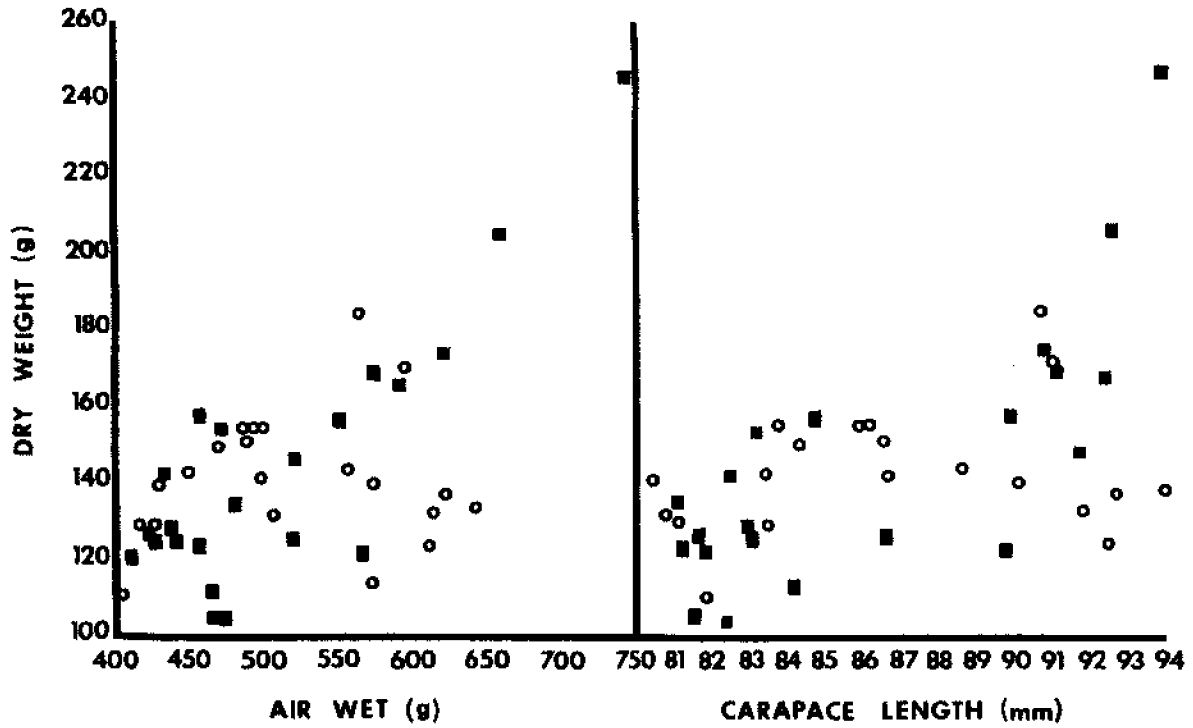


Figure 1. Scatter plots of lobster dry weight versus air weight and carapace length

■ = male ○ = female

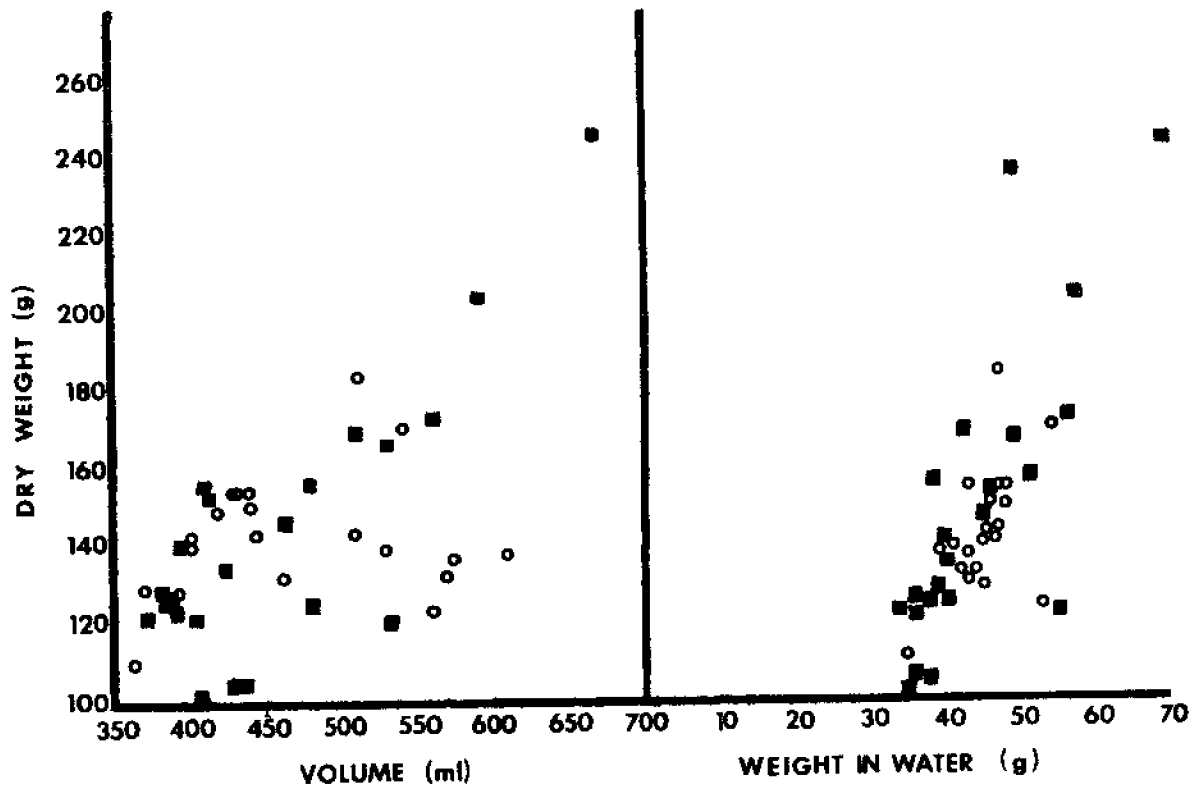


Figure 2. Scatter plots of lobster dry weight versus volume and weight in water

■ = male ○ = female

SUMMARY

Coordination of research and communication among researchers and funding of lobster nutrition were seen as major problem areas. It was suggested that some form of joint project be proposed to encompass most aspects of lobster aquaculture. A national lobster nutrition project was proposed, with each research unit having tasks to be specified as part of the overall project. Although the Canadian researchers would not be funded as part of this proposed project, it was agreed that they should coordinate with it.

Dr. Gallagher accepted responsibility for program development of the National Lobster Nutrition proposal.

There was discussion on standardization of research methods and the use of standard reference diets. A reference diet would better enable comparison of growth results in lobsters reared at various laboratories; crab protein was suggested as a possible reference.

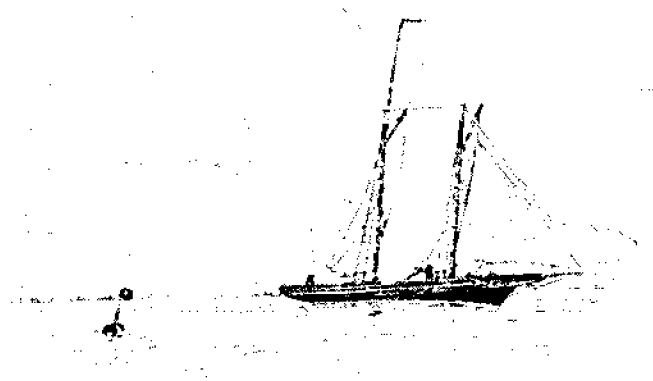
This was the first meeting of all those research groups presently involved in lobster nutrition research with presentation and discussion of their most recent work. The idea of gathering together in a small group environment yielded a worthwhile endeavor and should happen more frequently, perhaps annually or semi-annually.

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