PROJECT COMPLETION REPORT

96-5 "Dietary and Hormonal Regulation of Intestinal Phosphate Absorption in Fish"

Termination Report Period: June 15, 1996 through September 30, 1998

NRAC Total Funding: $116,810 (June 15, 1996 through June 14, 1998)
(No-Cost Ext'n through to September 30, 1998)

Principal Investigator: Dr. Ronaldo P. Ferraris, New Jersey Medical School

Participating Investigators/ Cooperative Agencies:

Dr. Enrique Avila	New Jersey Medical School	New Jersey
Dr. Pedrick Weis	New Jersey Medical School	New Jersey
Mr. Mike Hendrix	Aquaculture Research Facility, USFWS	Pennsylvania

---

Project Objectives:

This project focuses on a novel method to reduce phosphorus levels in effluents from aquaculture by using dietary and hormonal manipulations to improve intestinal absorption of phosphate. Its long-term goal is to reduce total and inorganic phosphorus levels in effluents from aquaculture. Its specific aim is to reduce feed and fecal phosphorus content by dietary and hormonal induction of intestinal phosphate transport. This project will initially focus on a freshwater species, *Onchorhynchus mykiss* or rainbow trout.

To achieve these goals, the project specifically seeks to:

1. Determine the mechanism of intestinal phosphate absorption in isolated, everted sleeves of rainbow trout small intestine.
2. Determine whether changes in levels of dietary phosphate modulate intestinal phosphate transport;
3. Determine the type of dietary vitamin D that is most effective in enhancing intestinal phosphate transport;
4. Determine the effect of changes in levels of dietary vitamin D_3 on intestinal phosphate absorption and on plasma levels of 25-(OH) vitamin D_3, 1,25-$(OH)_2$ vitamin D_3, and phosphorus; and,
5. Combine the most promising vitamin D metabolite (objective 3), vitamin D level (objective 4) and phosphate (objective 2) concentrations and determine their synergistic effect on intestinal phosphate absorption and phosphate assimilation.

Anticipated Benefits:

Further development of aquaculture in the US has been hindered, in part, by its potentially negative impact on the environment. A critical problem associated with intensive aquaculture production is the generation of aquaculture waste emanating from excess, uneaten food, or from unabsorbed nutrients in the fecal matter. Phosphates and nitrates constitute the main pollutants in effluents from aquaculture; a modest hatchery or fishpond operation can easily discharge over a ton of phosphorus a year. To prevent rapid eutrophication of nearby lakes and rivers, environmental regulatory agencies are now making stringent guidelines to limit the amount of potential pollutants the aquaculture industry can discharge into public waters. These environmental guidelines not only inhibit the expansion of the aquaculture industry but also pose a serious problem for existing hatcheries and farms. Failure to lower phosphorus levels in effluents is therefore a major bottleneck in production of aquacultured fish.

This project not only offers a novel practical approach towards improving intestinal phosphate absorption using dietary components already present in fish diets, but also improves our understanding of this hitherto neglected aspect of mineral nutrient homeostasis. While there are a large number of studies on calcium metabolism by fish, no study of dietary phosphate and vitamin D regulation of phosphate absorption has been done on fish. Thus, if the specific aims of this project are achieved,

information from results will lead to increased understanding of the role played by intestinal absorption or malabsorption in the generation of feed-derived pollutants. Results will also provide some of the database for use in designing diets that can reduce phosphorus content in aquaculture effluents.

Principal Accomplishments:

The major accomplishments of this project are results which :

- demonstrate that a phosphate (P_i) transporter is found in trout small intestine
- indicate that this transporter is not related to the sodium phosphate transporter cloned from flounder kidney
- suggest that the trout intestinal phosphate transporter is not regulated by dietary phosphate levels
- suggest that the trout intestinal phosphate transporter is not regulated by dietary vitamin D levels
- define the aquaculture factors (experimental diets, fish density, flow rate, influent P_i concentrations, effluent P_i concentrations) that would be optimal for measuring effects of dietary vitamin D and phosphorus levels on aquaculture effluents.
- suggest that high vitamin D, perhaps acting through other metabolic pathways, reduces levels of phosphate in aquaculture effluents

These accomplishments indicate that we have achieved all objectives outlined in the grant proposal. The results are now summarized briefly; a more detailed and technical summary is provided in the "Technical Report" section and in the appendix.

<u>An intestinal phosphate transporter is found in rainbow trout</u>.

We have now unequivocally shown by physiological methods that intestinal P_i absorption is carrier-mediated. For 44 g trout, we found the maximum transport rate (V_{max}) and substrate affinity (K_t) to be 0.21 nmol/mg min and 5.7 mM, respectively. In 12 g trout, the kinetic constants of the saturable mechanism were very similar (K_t = 7.54 mM and V_{max} = 0.35 nmol/mg min), indicating that the P_i transporter do not change with age or size. Trout intestine therefore exhibits a saturable P_i transport, indicative of a carrier.

These findings have been supported by competitive inhibition tests. Uptake of tracer ^{32}phosphorus (1.18 mM) was completely inhibited in the presence of a higher concentration of non-labeled phosphate (20 mM), suggesting further that P_i transport in trout intestine is carrier-mediated. Next, we incubated trout intestines in either the presence or absence of sodium (Na^+), and with or without phosphonoformic acid (PFA, 1 mM), a specific inhibitor of P_i transport in mammals. Results showed that P_i transport decreased by 90% in the absence of Na^+, and 80% in the presence of PFA, strongly indicating that intestinal phosphate transport in trout is Na^+-dependent and carrier-mediated.

We also found no significant difference in P_i transport between the proximal and middle intestines, but it was significantly lower in the distal region. The distal region was not used in all other uptake measurements.

We then demonstrated striking differences between P_i concentrations in the plasma and in the intestinal lumen of trout fed commercial chow. There is a much higher P_i concentration in the plasma (5.5 mM) than in the luminal fluid (1.0 mM), presenting a problem for trout because intestinal Pi needs to be absorbed against a 5X concentration gradient. A Na^+-dependent phosphate transporter has been biochemically demonstrated in the winter flounder. While our initial results suggested that the trout intestinal phosphate transporter may be related to the flounder phosphate transporter, several subsequent experiments indicated that the two transporters may be biochemically unrelated despite some functional similarities.

<u>Intestinal phosphate transport is not regulated by dietary phosphorus levels</u>.

The experimental diets contained different levels of dietary phosphate: 0.035% (available phosphate from egg white only (the protein source), 0.1%, 0.3%, 0.58%, 1.2% and 2.4% (available phosphate from egg white plus additional monobasic phosphate). Please see Table 1 for components of the purified diet, which contains 0.6% P_i and 3000 IU/kg vitamin D. These were then fed to trout, first, in 2 experimental series, one lasting 7 days, and another over 28 days. At the conclusion of the feeding period, trout was sacrificed, their intestines removed and P_i uptake measured. Likewise, phosphate concentrations in the blood and intestinal contents were determined.

Intestinal P_i uptake did not vary among trout fed varying levels of dietary phosphate, regardless of the length of the feeding period. In contrast, plasma and intestinal luminal P_i concentrations varied significantly with dietary P_i in the 7- and 28-day experiments. These differences can not be accounted

for by differences in the amount of food consumed since food consumption among treatment groups was similar. These results suggest that, unlike mammals, dietary phosphate does not regulate intestinal P_i uptake. These also suggest that the amount of P_i consumed affects P_i concentration in intestinal contents and in the plasma. By affecting plasma P_i concentration without affecting intestinal P_i absorption, other physiological processes must be regulating P_i metabolism. These results have critical implications on the interrelationships among intestinal P_I transport, plasma P_i regulation, vitamin D action and the current levels of P_i in commercial trout feeds.

Intestinal phosphate transport is not regulated by dietary vitamin D levels

Phosphorus requirement in trout is high but retention is reportedly low owing to high rates of metabolic or fecal losses. Since the vitamin D endocrine system modulates calcium and phosphorus homeostasis, we determined the effects of increasing concentrations of dietary cholecalciferol on intestinal inorganic phosphate (P_i) uptake, plasma P_i, Hepato-Somatic Index (HSI, indicative of liver size) and circulating vitamin D_3 metabolites in trout fed P_i-sufficient diets (6 g/100 g). Five groups of 24 trout initially weighing 55.8±0.58 g were fed purified diets containing 0, 300, 2500, 10000, and 40000 IU vitamin D_3/kg diet over a 7-day feeding period. While increasing levels of dietary cholecalciferol did not enhance in vitro intestinal P_i uptakes, these significantly increased plasma P_i and reduced the HSI without any apparent change in circulating $25(OH)D_3$ and $1,25(OH)_2D_3$. Mean intestinal uptakes ranged from 0.19-0.28 nmoles/mg min across all dietary levels. Tissues were also incubated directly in solutions containing various vitamin D metabolites, but none had an effect on intestinal P_i transport. Plasma P_i was higher in trout fed over 300 IU/kg diet, with a grand mean of 8.26±0.27 (n = 15) mM. The HSI (2.05) was significantly higher in fish fed 0 IU/kg. These results demonstrate that dietary cholecalciferol increases plasma P_i concentrations but decreases the HSI. Diet-induced changes in plasma P_i concentrations and HSI are not correlated with changes in intestinal P_i uptake.

Effect of dietary phosphorus and vitamin D on P_i utilization by juvenile trout and on levels of P_i in the effluent

This project was done with scientists from the Northeast Fishery Research Center (US Fish and Wildlife Service), Lamar, PA from May to Jul. 1998. The main purpose of this preliminary work was to define the parameters that would be optimal in measuring effects of dietary vitamin D and phosphorus levels on aquaculture effluents. Some analysis of this experiment is still ongoing.

We have concluded that feeding rates of 1 - 2% biomass per day, that flow rates of 6 - 8 l/min, and that 25 kg of fish cultured in 400 - 500 l volume of water are sufficient to detect effluent phosphorus concentrations. For effluent samples, we also determined the appropriate sampling conditions, sample preservatives, and method of analysis. For fish samples, we resolved the drying and ashing conditions appropriate for determinations of body P_i composition. We also found that our experimental diet which proved highly useful in laboratory experiments, turned out to be vastly inferior to commercial chows in palatability, leading us to slightly modify our diet in order to simulate commercial farming conditions as closely as possible. These preliminary results will now be used for the main effluent P_i experiments to be conducted in the 1998 - 2000 phase of this project.

Growth and survival

Juvenile rainbow trout were fed purified diets containing different levels of phosphorus (0.3 or 6 %) and vitamin D (2500 or 10000 IU per kg). Fish fed a commercial trout diet (1.2% Pi, 800 IU/kg vitamin D) served as a control. Mean weight gain was significantly higher in fish fed the commercial diet (Diet 4) followed by fish fed the diet containing 0.6% P and 2500 IU vitamin D (Diet 1). Fish fed the diets with 0.3 % and either 2500 or 10000 IU (Diets 2 and 3, respectively) had the lowest mean weight gain and did not differ significantly from each other. Survival was high (about 98%) and was similar in all the treatments.

Carcass dry mater, ash, and phosphorus contents

Fish carcass contents of dry matter and ash (on dry matter basis) were not significantly different in initial and final samples obtained from the various treatments. The phosphorus contents of initial fish samples were similar in all the treatments. However, at the end of the experiment, fish fed the commercial diet had significantly lower phosphorus content on a dry matter basis (2.18 %) compared with those from the other diets (2.37 - 3.81 %).

Phosphorus concentration in effluent

The total phosphorus concentration of the effluent water was also measured on three occasions (at days

8, 9 and 16 of rearing) every two hours from 0800 h to 1800 h. The values used were net values after the phosphorus concentration of the inlet water was subtracted from the measured phosphorus concentration of the sample obtained at the same time point. In two of three sampling days, diet effects were clearly significant as effluents from tanks with fish fed the commercial diet (high Pi, lowest vitamin had the highest phosphorus concentrations. The lowest values observed were those in fish fed the diet containing 0.3% P and 10000 IU/kg vitamin D. The effluent phosphorus concentrations over time did not differ significantly among the treatments. However, two peaks in phosphorus concentrations of water samples obtained after feeding were clearly evident at 1000 h and after 1400 h, which corresponded to the sampling times around feeding.

Impacts:

The physiological mechanism of intestinal phosphate absorption has, for the first time, been elucidated in fish. Although the presence of this carrier implies that phosphate transport can be regulated biologically, we were not able to demonstrate biological regulation of phosphate transport by dietary phosphate or vitamin D in the small intestine. Nevertheless, a high vitamin D, in combination with low phosphate concentrations in the diet, significantly decreased effluent phosphorus concentrations during preliminary experiments in fish culture tanks. Hence, we suspect that biological regulation may be effective in other physiological processes (e.g. phosphatases) or other organ systems (e.g. renal or branchial (gill) P_i transport).

Recommended Follow-Up Activities:

(to be conducted in 1999 and 2000)

Conduct full-scale experiment evaluating effect of dietary phosphate and vitamin D on concentrations of phosphate in the effluent and fecal matter of fish.

Determine growth, survival, phosphate utilization, intestinal phosphatase activity, plasma phosphate and vitamin D concentrations in this fish. Shift from purified diets to semi-purified and practical diets to simulate commercial conditions

Publications, Manuscripts Or Papers Presented:

Avila, E.M., S.P. Basantes and R. P. Ferraris. Manuscript submitted to General and Comparative Endocrinology, is presently under revision. Title: "Cholecalciferol modulates plasma phosphate but not plasma vitamin D and intestinal phosphate absorption in rainbow trout *Onchorhynchus mykiss*."

An abstract, entitled "Mechanisms of Phosphate Transport by Trout Intestine," has been published by American Zoologist, a publication of the Society for Integrative and Comparative Biology. Dr. Avila was invited to give a stage presentation of results from this project in its annual meeting on January 3-7, 1998 in Boston, MA.

Manuscript in preparation: Intestinal phosphate absorption in rainbow trout *Onchorhynchus mykiss*.

TECHNICAL ANALYSIS AND SUMMARY

(Note: we have generated a lot of data, and only figures and tables of main or interesting findings are presented)

Objective 1 Determine the mechanism of intestinal phosphate absorption in isolated, everted sleeves of rainbow trout small intestine.

Results:

For the series of experiments on uptake mechanisms, the mean body weights of fish used were: 53.8 ± 14.2 g (n = 7) for regional effects (**a**); and, 64.2 ± 12.9 g (n = 16) for optimum incubation time (**b**). In determining kinetic parameters (**c**) we used two sizes: 50.9 ± 10.7 g (n = 25), and 10.9 ± 2.7 g (n = 39), representing the medium-sized (~ 6 mos. older) and small-sized classes, respectively. Fish used for the inhibition tests had body weights of 127 ± 25 g (n = 5), self-inhibition (**d**); 13.2 $\pm$ 2.9 (n = 24), for uptakes under Na-free conditions (**e**); and 12.2 ± 2.6 g (n = 9) (**f**), competitive inhibition.

Effect of P_i Concentration in Incubation Medium on P_i Uptake (this result shows that the uptake mechanism is saturable and therefore carrier-mediated and regulatable)

In 12 g fish, intestinal P_i uptakes in Na^+-free and Na^+-containing are each proportional to the P_i concentration in the incubation medium (please refer to Fig. 1). Uptake rates under Na^+-free conditions increase in a linear fashion with increasing P_i concentrations in the medium (open triangles). The diffusion coefficient (K_d) of this Na-independent uptake is 0.0124 min^{-1}. Uptake rates under Na^+-containing conditions (or total uptake rates, open circles) tend to be hyperbolic and non-saturable. Subtracting Na^+-free P_i uptake rates from total uptake rates yields a saturable mechanism of uptake (filled triangles). The kinetic parameters of the saturable

mechanism determined by Michaelis-Menten nonlinear regression were K_t 7.54 mM and V_{max} 0.35 nmoles/mg min. Similar results were obtained for the intestines of 44 g trout, with K_t and V_{max} of 5.7 mM (P = 0.18) and 0.21 nmoles/mg min (P = 0.38), respectively.

Competitive Inhibition: (this result shows that the uptake mechanism must be carrier-mediated because absorption of radiolabelled phosphate can be blocked by phosphate analogs or by unlabelled phosphate and that it must be Na^+-dependent and therefore active because it is inhibited by Na^+-free solutions)

Uptake of tracer $^{32}P_i$ (1.18 mM) was almost completely (>90%) inhibited in the presence of high concentrations of non-labelled P_i (20 mM) (P<0.0001) (Fig. 2a). Uptake of 0.22 mM P_i was also strongly (75%) inhibited by the presence of PFA (P<0.001) and decreased by 88% in the absence of Na^+ (P<0.001) (Fig. 2b). Uptake in the absence of Na^+ and in the presence of PFA were not significantly different (P = 0.55).

Objective 2 Determine whether changes in levels of dietary phosphate modulate intestinal phosphate transport.

Results:

Effect of Dietary P_i Level on Intestinal P_i Uptake, Plasma and Luminal Fluid P_i Concentration, Plasma P_i concentration of trout (12.2 g) fed commercial trout chow (1.3% Pi) was 5.5 ± 0.7 mM (n = 4) and the luminal fluid P_i concentration was 6.6 ± 1.1 (n = 4), and were not significantly different (P = 0.49).

Intestinal P_i uptake did not vary among trout fed varying levels of dietary phosphate, regardless of the length of the feeding period (Figs. 3 and 4, refer to filled bars). Intestinal proline uptake (open bars) was used as control not only to indicate viability of the tissue, but also to demonstrate that P_i uptake, if it changes, would specifically respond to changes in dietary P_i levels.

In contrast to P_i uptake rates, plasma and intestinal luminal P_i concentrations varied significantly with dietary P_i in the 7- and 28-day experiments. In the 7 day experiment (Fig. 5), luminal fluid P_i concentration (open bars) increased with dietary P_i concentration, with the most increase occurring between 1.2 and 2.4% dietary P_i concentration (P = by one way ANOVA). Linear regression analysis revealed a significant slope which indicated that luminal fluid increased by 7.4 mM for each percent increase in dietary P_i concentration. The predicted luminal P_i concentration for a P_i free diet is 2.48 mM; this suggests that trout would still excrete P_i even in the absence of dietary P_i. In contrast, plasma P_i concentration (filled bars, Fig. 5) was tightly regulated between 0.035 - 1.2% dietary Pi, increasing only modestly when dietary P_i concentration reached 2.4%. Linear regression analysis indicated a modest increase of 1.75 mM in plasma P_i concentration for every percent increase in dietary P_i level; this slope is significantly different from 0 (P = 0.03). The predicted plasma P_i concentration at 0% dietary P_i is 4.5 mM. The correlation coefficient for plasma and dietary P_i is 0.37 which is much lower than that for luminal fluid and dietary P_i (0.64), indicating that other factors are more important than dietary P_i level in regulating plasma P_i concentrations.

For fish fed experimental diets for 28 d, intestinal luminal fluid P_i concentration was generally very low from 0.035 to 0.6% dietary P_i (Fig. 6, open bars), but was followed by sharp increases in luminal P_i at 1.2 and 2.4 % dietary P_i (P = 0.01). These sharp increases is reflected in a steeper slope: for each % increase in dietary P_i level, luminal fluid P_i concentration increased by 13.2 mM. This slope is significantly greater (P = 0.02) than that for 7 d fish. The predicted luminal fluid P_i concentration at 0% dietary P_i is 0 mM. Plasma P_i concentration was tightly regulated, although there is a modest increase at high dietary P_i levels (P = 0.03 by one-way ANOVA, Fig. 6, filled bars). Linear regression analysis indicated a modest increase of 1.54 mM for each percent increase in dietary P_i level; this slope is similar (P = 0.28) to that for 7 d animals. The predicted plasma P_i concentration at 0% dietary P_i is 4.46 mM, a concentration significantly different from 0 (P = 0.002) but not significantly different from that for 7 d animals.

What is the relationship between luminal fluid and plasma P_i concentrations? Correlation analysis again suggest a tight regulation of plasma P_i concentrations. For the 7 d experiment, plasma P_i concentration changes by 0.18 mM for every mM change in intestinal luminal P_i concentration. The slope decreases (P <) by 50% if trout is allowed 28 d to adapt to the diet: 0.09 mM for every mM change in luminal Pi. The intercept increases with duration of feeding. The predicted plasma P_i concentration at 0mM luminal P_i concentration is 4.38 mM at 7 d, and 5.20 mM at 28 d.

Objective 3 Determine the type of dietary vitamin D that is most effective in enhancing intestinal phosphate transport.

Results:

Intestinal P_i Uptake in Tissues Incubated in Different Vitamin D Metabolites Intestinal P_i uptake by trout is independent (P = 0.40) of the types of vitamin D metabolites in the incubation medium (Fig. 7a). The average P_i uptake rate (Fig. 7b) in all treatments was 0.20 $\pm$ 0.01 nmol/(mg·min). Proline uptake rate was also independent of the types of vitamin D metabolites in the incubation medium. The average proline uptake rate in all treatments was 1.8 $\pm$ 0.07 nmol/(mg·min). These results demonstrate that P_i uptake is independent of vitamin D even in intestinal tissues directly pre-incubated in vitamin D solutions.

Objective 4 Determine the effect of changes in levels of dietary vitamin D_3 on intestinal phosphate absorption and on plasma levels of 25-(OH) vitamin D_3, 1,25-$(OH)_2$ vitamin D_3, and phosphorus.

Results:

Effect of Dietary Vitamin D on Intestinal P_i Uptake, Plasma Vitamin D, Plasma Pi, and Luminal Fluid P_i Concentration

Please refer to the attached manuscript (submitted to General and Comparative Endocrinology) for this section, and to the brief summary in pages 3 and 4 of this report.

Objective 5 Combine the most promising vitamin D metabolite (objective 3), vitamin D level (objective 4) and phosphate (objective 2) concentrations and determine their synergistic effect on intestinal phosphate absorption and phosphate assimilation.

The purpose of this specific aim is to apply laboratory findings to the field. Although dietary phosphate and vitamin D unfortunately had no effect on intestinal phosphate absorption, these had effects on plasma phosphate concentrations, indicating their effect on phosphate metabolism. Hence, we decided to go ahead with the experiments determining the effect of dietary phosphate levels and vitamin D on effluent phosphate levels. Moreover, by the time we found out that several critical laboratory findings were negative (no effect of dietary phosphate and vitamin D on intestinal phosphate transport), we already were already well underway for conducting experiments under Objective 5.

Results:

Growth and survival (to show survival and growth in experimental diets compared to commercial chow).

Juvenile rainbow trout were fed purified diets containing different levels of phosphorus (0.3 or 6 %) and vitamin D (2500 or 10000 IU per kg). Fish fed a commercial trout diet (1.2% Pi, 800 IU/kg vitamin D) served as a control. Mean weight gain was significantly higher in fish fed the commercial diet (*Diet 4, see Table 2*) followed by fish fed the diet containing 0.6% P and 2500 IU vitamin D (*Diet 1*). Fish fed the diets with 0.3 % and either 2500 or 10000 IU (*Diets 2 and 3*, respectively) had the lowest mean weight gain and did not differ significantly from each other. Survival was high (about 98%) and was similar in all the treatments.

Fish carcass contents of dry matter and of ash (when expressed as % dry matter) were each not significantly different in initial and final samples obtained from the various treatments. The phosphorus contents of initial fish samples were similar in all the treatments. However, at the end of the experiment, fish fed the commercial diet had significantly lower phosphorus content on a dry matter basis (2.18 %) compared with those from the other diets (2.37 - 3.81 %).

Phosphorus concentration in effluent

The total phosphorus content of the effluent water was also measured on three occasions (at days 8, 9 and 16 of rearing) every two hours from 0800 h to 1800 h. The values used were net values after the phosphorus content of the inlet water was subtracted from the measured phosphorus concentration of the sample obtained at the same time point. The set of values analyzed within a single day was then subjected to analysis of variance (ANOVA) with repeated measures to examine the dietary effects as well as the interaction effect over time. On day 8, our first attempt at sampling effluents, no significant differences were obtained when either diet or time effects were analyzed (data not shown). However, two peaks in phosphorus concentrations of the effluent were observed at about 1000 h and 1400 h which corresponded to the sampling times right after feeding. In Figs. 8 and 9 (sampling at days 9 and 16, respectively), the diet effects were clearly significant in these two occasions at $p<0.05$ but the interaction effect over time remained insignificant. In both sampling days, effluents from tanks with fish fed the commercial diet had the highest phosphorus concentrations. Effluents from tanks with fish fed the purified diets had lower concentrations of phosphorus with the lowest values observed in those with fish fed the diet containing 0.3% P and 10000 IU/kg vitamin D. However, only the values from Diet 4 were

significantly different from all other values. Furthermore, the phosphorus concentrations over time did not differ significantly among the treatments on both occasions. However, two peaks in phosphorus concentrations of water samples obtained after feeding were clearly evident at 1000 h and after 1400 h.

This last experiment demonstrates the potential of reducing P_i levels in aquaculture effluents by dietary and hormonal manipulations.

The Full Report is available at the NRAC office upon request.