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Pyceze™ assists larval survival in lobster culture

ABSTRACT

The performance of a new prophylactic chemical product, Pyceze™, was assessed at the National Lobster Hatchery (NLH), Cornwall. An optimum dose for Pyceze™ application to Artemia nauplii was determined to be 150mgL^{-1}. A 10 day trial was carried out in two recirculation systems (Control and Test) to examine the effect, on larval mortality rates, of feeding Pyceze™-treated Artemia to larvae of the European lobster, *Homarus gammaurs*. Counts of heterotrophic and *Vibrio* species bacteria were reduced throughout the trial in the Test system. Mortality of the Test larvae was reduced by 30% from Control losses (chi-square tests shown to be highly significant ($P < 0.01$)). It was concluded that the use of Pyceze™ by the NLH was fully justified as a dietary disinfectant and that the applicability of the product for use in the culture of other species could be extensive.

INTRODUCTION

Results of testing at the National Lobster Hatchery (NLH) in Padstow, Cornwall, provide evidence that the prophylactic treatment Pyceze™, marketed in the U.K for commercial aquaculture by Novartis© Animal Vaccines, was able to reduce mortality rates of larval European lobsters, Homarus gammarus, when added as a disinfectant to the culture of Artemia nauplii feed. Pyceze™ bactericidal efficacy is achieved by a 50% solution of the chemical Bronopol (2-bromo-2-nitropropane-1,3-diol). Though developed specifically to be a fungal treatment for the salmonid industry, Pyceze™ has been found to be successful in reducing mortality in many marine animals, including other fish and juvenile scallops. This is thought to be the first trial of the chemical with larval lobsters.

The NLH runs an established project designed to culture and release juvenile animals to enhance the regional population of *H. gammarus*. By conducting the larval stages of development in the hatchery, the lobsters avoid the wild planktonic phase, during which mortality levels are significant. The stock enhancement work aims to significantly improve the sustainability of the *H. gammarus* fishery in Cornwall and the Isles of Scilly. The program relies upon the fact that *H. gammarus* displays high natural larval mortality and low juvenile and adult mortality. Therefore increasing larval survival rates at the NLH increases the efficiency of the project as a whole.

Much mortality among the larvae is classed as unavoidable (e.g. larval cannibalism and intra-population aggression). However, considerable mortality still occurs as a result of 'avoidable' trauma, such as infection by viral, fungal or bacterial diseases. Although disease problems occur in the wild, epidemia in hatchery conditions can be very problematic.

Early-stage *H. gammarus* larvae have a greater chance of successful development if fed on live food. However, the cultivation of *Artemia* nauplii feed in the hatchery allows extensive growth and diversification of the microbial community within its culture water. By ascertaining the degree of microbial colonization in a variety of hatchery locations, the likely source of pathogenic

This article describes work carried out at the National Lobster Hatchery, South Quay, Padstow, Cornwall, and in partnership with the University of Plymouth, by C.D. Ellis, S. Davies, D. Boothroyd, D.J. Taylor, R.T.A. Pryor, C. Daniels. For further information please see www.nationallobsterhatchery.co.uk, or contact Charlie Ellis on tel: +44 (0)1208 73230, or email: info@nationallobsterhatchery.co.uk

introductions could be identified for further isolation and treatment. INVE© BDS-G™ dip slides incorporating specialised marine agar were used to determine concentrations of heterotrophic and *Vibrio* spp. bacteria in cultures of *Artemia* nauplii and *H. gammarus*, and in the hatchery seawater supply tank. As predicted, the live feed culture had by far the highest bacterial loading, especially of the pathogenic *Vibrio* spp.

MATERIALS AND METHODS

Prior to embarking on the main larval investigation, the optimum concentration of Pyceze™ application to the *Artemia* culture was determined. Bronopol at 150mgL^{-1} (300 mgL^{-1} of Pyceze™, the highest dose administered during tests) was determined to be the most promising concentration, providing an 89.4% reduction in the population of *Vibrio* spp. without significant *Artemia* mortality.

For the larval lobster trials, two (approx.) 150 litre re-circulation systems were assembled to simulate the hatchery's own conditions, each with a pair of 33 litre kreisel cones containing approximately 750 larvae, suspended above an 85 litre sump vessel. Seawater flow rate in recirculation was 1060L/hr. *Artemia* were cultivated in 10L vessels, with 50,000 nauplii cultured at 20°C (±2°C) and 35‰ (±2‰). Both cultures were administered with 600mgL^{-1} of Selco™, 7.2mgL^{-1} of Bio-Moss™ and equal aeration, but, while the Control culture had no Pyceze™ added, the Test culture was supplemented with 3000mg of Pyceze™ (to yield a solution with 150 mgL^{-1} of Bronopol). The nauplii remained in their respective solutions at concentrations just below 150

nauplii ml^{-1} (approximately 300,000 nauplii per vessel) for 24 hours, until they were fed to the lobster larvae. Water quality parameters were measured throughout trialling. Salinity (36‰, ±2‰), Temperature (19.5°C, ±1.5°C), pH (8.0, ±0.2), Nitrite (0.0‰, ±0.4‰) and Ammonia (0.0‰, ±0.25‰) conditions between the systems were kept consistent.

Left - The NLH's *Artemia* culture cones. *Artemia* nauplii hatch from cysts into solution and are supplemented, using Selco™ enrichment and Bio-Moss™ immunostimulant, then disinfected with Pyceze™ before being harvested and fed to the larval lobsters at [5 mgL^{-1}]. Without disinfection, these cones could be colonized readily by harmful microbes.

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RESULTS

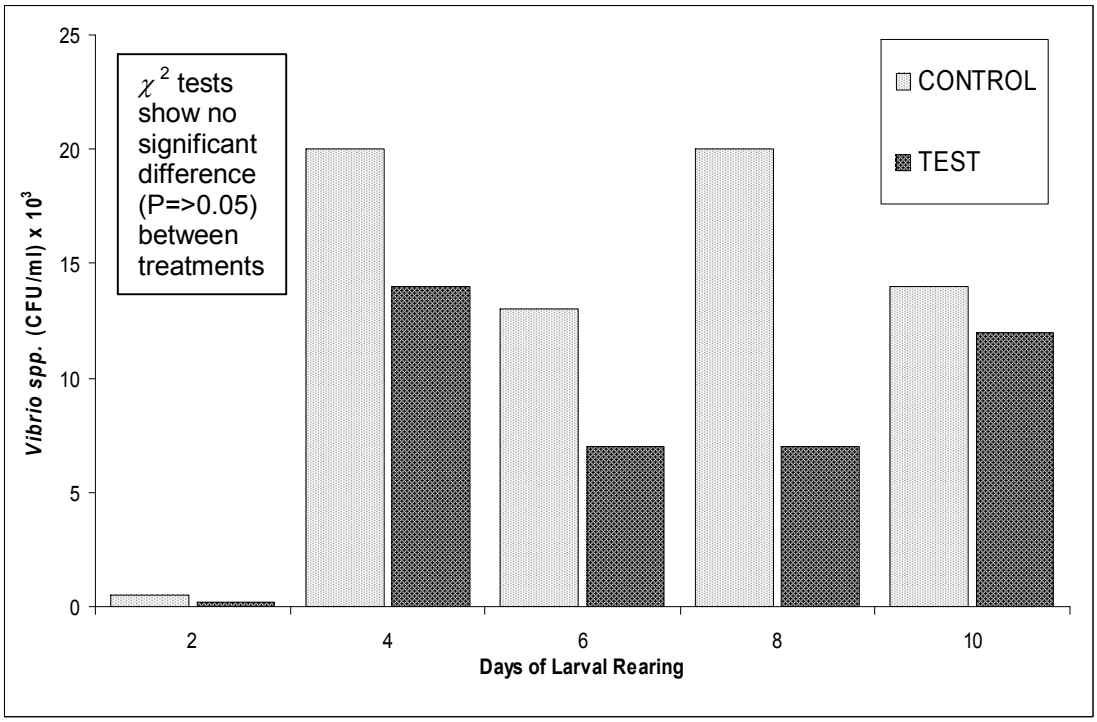

Figure 1 – A histogram showing fluctuations in the levels of *Vibrio* species bacteria within the culture systems throughout the 10 days of the investigation.

Figure 1 shows there was a distinct difference in the degree of bacterial loading between the Control and Test systems. *Vibrio* spp. CFU/ml was higher in the Control than the Test in every sample, and on Day 8 the Control system's *Vibrio* spp. concentration was almost triple the levels sampled from the Test system. So throughout the larval rearing trials, the degree of culture water contamination in the Control system was consistently greater than it was in the Test system.

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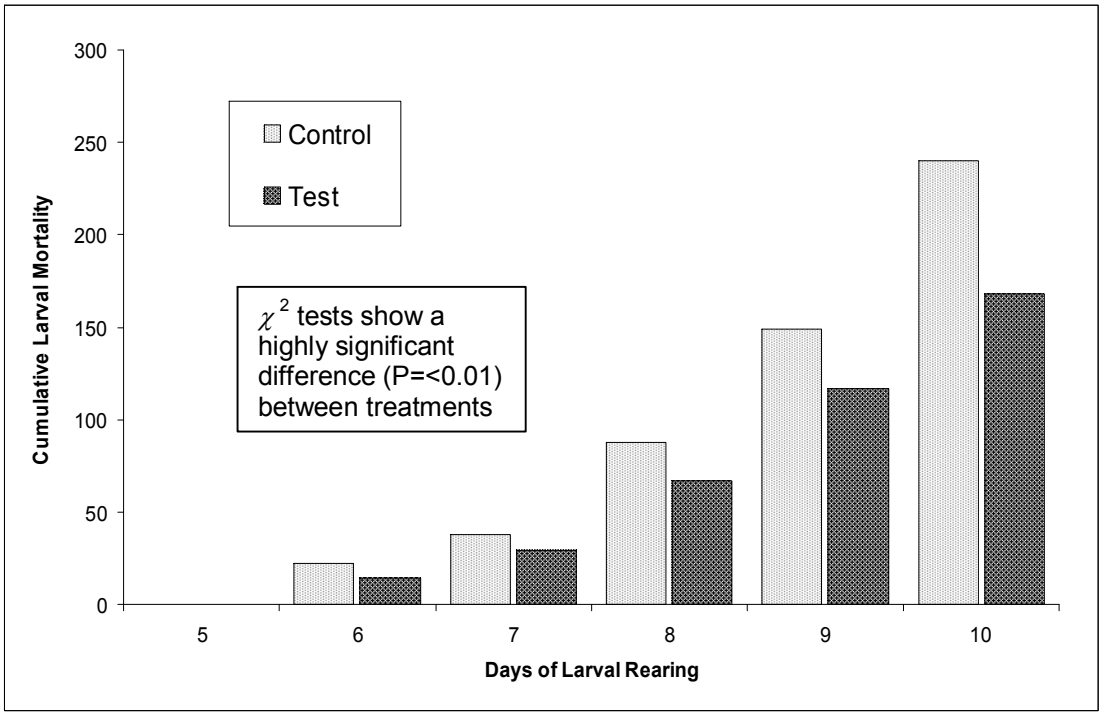

Figure 2 – A histogram showing the total cumulative *H. gammarus* larval mortality within the Control and Test systems throughout the days of the trial on which mortality occurred.

The level of larval mortality was consistently higher in the Control system than it was in the Test system, while the rate of increase in mortality also accelerates faster in the Control system, as can be seen in Figure 2 (above). Between Days 6 and 10, the level of cumulative mortality was consistently reduced by >22% in the Test system compared those for seen in the Control system. By Day 10 a total of 240 larvae had died from the Control system and 168 mortalities were recorded from the Test system, a difference of 72 individuals. This difference represents a 30% decrease in the occurrence of larval mortality in the Test treatment. Given that each system began with 1500 larvae, the overall survival rate to Day 10 was 84% for the Control and

89% for the Test. However, this assumes that no larvae were lost to cannibalism, whereas, at the stocking densities exhibited in this trial (almost 23 larvae/litre) the loss is likely to have been significant, so the difference between the treatments in overall survival rate is likely to be considerably greater.

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DISCUSSION

Vibrio spp. colonisation was reduced in the Test system in every count. This provides a strong indicator that Pyceze™ is effectively controlling the proliferation of pathogenic microbes, and the extent to which it is able to do this (Figure 1 shows that *Vibrio* colonisation in the Test is only 35% of the level seen in the Control on Day 8) suggests that Pyceze™ treatment is causing the observed reduction in larval mortality in the Test.

The difference between treatments in *Vibrio* colonisation was not found to be statistically significant ($P>0.05$) by χ^2 test though, even after the data had been naturally logged. However, this result could be misleading in terms of the ability of pathogens to cause death by the continual weakening and cumulative overpowering of the lobster's immune defence. Like most marine invertebrates, lobsters have reduced phagocytic activity of their haemocyte cells during larval stages and their ability to fight *Vibrio* spp. at concentrated levels during a first exposure is unspecific and severely limited. Over the course of the investigation's ten days, larvae in the Test tank were exposed to less than 60% of the amount of *Vibrio* spp. that Control larvae encountered. This may well have helped keep many Test larvae healthy enough to survive.

Of all the measured variables in the larval rearing investigation, the reduced bacterial population, particularly that of the genus *Vibrionaceae*, seems the most likely source of the statistically highly significant ($P<0.01$) larval mortality reduction in the Test. Although mortality doesn't correlate directly with the highest concentrations of *Vibrio* spp. bacteria, the difference in the rate of larval deaths between the two trials may still be attributed to the degree of bacterial colonisation. Days 4 and 8 show the greatest influx of heterotrophic and *Vibrio sp.* bacteria in the Control system, yet more larvae perished in both control cones on Day 10. Likewise, Day 4 saw the most concentrated colonisation of the Test system by both heterotrophic bacteria and *Vibrio* species, yet Days 9 and 10 saw the most larval mortality. This suggests that *H. gammarus* larvae were not killed outright or even directly by pathogens.

Mortality is likely to occur as a result of prolonged contact with pathogens, or because of continual weakening that leads to mortality during a stressful event, like handling, exoskeletal moulting between larval stages, or exposure to atypical nitrite, ammonia or salinity concentrations.

Overall this investigation provides a valid and encouraging assessment of Pyceze's™ performance as a prophylactic treatment in cultures of larval *H.gammarus*. Pyceze™ provides a valuable resource in disease deterrence and the prevention of larval mortality at the National Lobster Hatchery. Further, wider research into the effects of Pyceze™, encompassing all aspects of hatchery health management and product viability, can only help reveal its potential significance and applicability in stock replacement schemes and other aquaculture projects.

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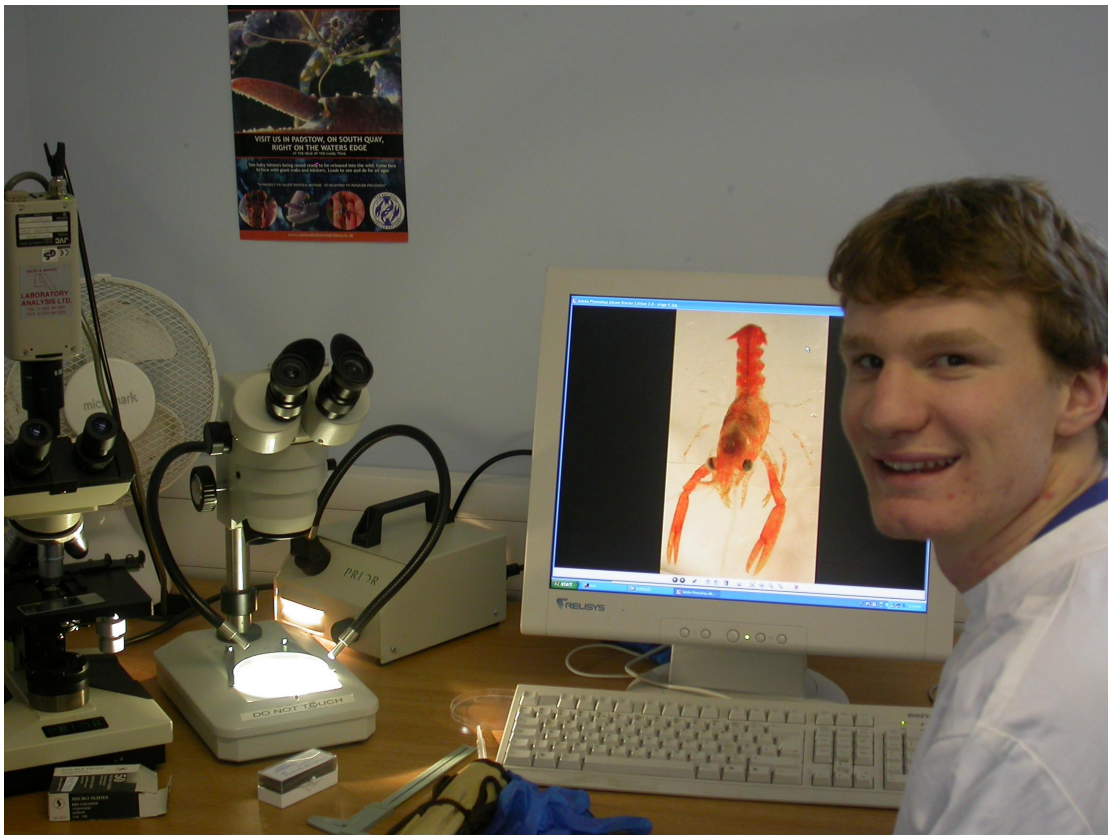

Undergraduate researcher Charlie Ellis.

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